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THE BLOOD SUPPLY OF THE HYPOPHYSIS IN *MACACA MULATTA*

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Views about the blood supply of the mammalian hypophysis have undergone many changes over the past three decades, and descriptions to-day derive largely from the review of the subject that was published by Green in 1951. A prominent conclusion of his survey is that the adenohypophysis, and in particular, the pars distalis, has a blood supply which is independent of that of the neurohypophysis. The general belief is that blood passes into the sinusoids of the pars distalis by way of portal vessels which begin in a primary capillary plexus in the median eminence, which is in turn fed by the superior hypophyseal arteries; and that the vascular network of the neural process is fed separately by a pair of inferior hypophyseal arteries. The venous blood from both parts of the gland passes *via* short veins into the surrounding venous sinuses.

Recent work by McConnell (1953) and Xuereb, Prichard & Daniel (1954*a, b*) on the human hypophysis qualifies this picture in so far as it indicates that there is a constant anastomosis between the superior and inferior hypophyseal arterial systems, and therefore between the blood vessels of the pars distalis and those of the neural process. The latter workers claim that the superior hypophyseal artery of each side, which arises from the internal carotid, gives branches to the median eminence and upper part of the pituitary stalk ('upper infundibular stem' in their terminology), and that it also gives off an 'artery of the trabecula' (McConnell's 'lateral artery'), which runs through the pars distalis to supply the lower ('intraglandular') part of the stalk ('lower infundibular stem'). The latter, however, is also fed by the inferior hypophyseal arteries, which spring from the internal carotids and which branch to form an arterial ring around the neural process. From this ring branches are given off to the tissue of the neural process, and also to part of the lower infundibular stem, where anastomoses with the superior hypophyseal system occur.

No branches of either set of arteries are said to supply the epithelial tissue of the human pars distalis directly. The branches of the superior and inferior hypophyseal arteries which run to the pituitary stalk break up to form a capillary network which is drained by 'long' and 'short' portal vessels, through which blood passes into the sinusoids of the gland. The 'long' vessels drain the upper, and the 'short' the lower part of the stalk, and in the human these two sets of vessels are described as the only afferent channels to the sinusoids of the pars distalis. The 'short' vessels supply that part of the glandular tissue which lies adjacent to the 'lower infundibular stem'. McConnell noted that a small wedge of pars distalis lying in front of the lower pituitary stalk is supplied both with superior and inferior hypophyseal blood, as are also a few small subcapsular areas of the gland.

This pattern of vasculature does not apparently apply to all mammals, although

Daniel & Prichard (1957*a*) found an essentially similar arrangement in the sheep. In the rabbit, Harris (1947) describes a direct arterial supply to the anterior lobe, as well as a pituitary portal system. A dual arrangement of this kind is also said to hold in the case of the macaque (Wislocki & King, 1936; Wislocki, 1938).

Conflicting views have been expressed about the possibility of an anastomosis between the sinusoids of the pars distalis and the vascular bed of the neural process. A connexion between the two would obviously be restricted in aquatic mammals such as the whale, where a fibrous septum intervenes between the adeno- and neurohypophysis (Wislocki & Geiling, 1936), and possibly also in animals such as the cat, where an intraglandular cleft persists between the pars distalis and pars intermedia. On the other hand, experiments which have been carried out on the rabbit by Brooks & Gersh (1940), and on the dog by Jewell (1956), suggest that there is a relatively free anastomosis in these two species. The former authors severed all arterial branches to the pituitary gland except the posterior lobe arteries, and found that injected material still entered that part of the pars distalis adjacent to the pars intermedia. Jewell's observations were made on the living animal, and he, too, found that blood passed very readily to the anterior lobe by way of the posterior lobe artery.

The present paper is concerned with the possibility that the anatomical basis of a similar anastomosis also exists in the monkey. The observations which are reported here were made during the course of a physiological study of the effects of section of the pituitary stalk.

EXPERIMENTAL

An attempt was made to divide the pituitary stalk of eight female monkeys (*Macaca mulatta*) for the primary purpose of studying the effect of this procedure on the menstrual cycle. The approach was by a temporal route. After division of the stalk, a Polythene plate was inserted between its cut ends. One animal died soon after the operation. Of the remainder, one died after 5 months, and the rest were killed at periods varying from 6½ to 18½ months after operation.

After death the vascular system of the forepart of each animal was perfused with a solution of indian ink, and after fixation in formol-saline, a block of tissue comprising the hypothalamus, sella turcica and pituitary gland was removed and decalcified. This block was later dehydrated, embedded in Necoloidine, and sectioned serially at 50 or 100 μ in a sagittal plane. The sections were stained with cresyl violet. In the later animals of the series, some thinner sections were cut and these were stained by a modified Cleveland & Wolfe's (1932) method.

Full details of the functional effects of stalk section in these animals will be published elsewhere, and the present report is concerned only with the effect of the lesion on the structure of the pars distalis, and on the possibility of vascular connexions between the pars distalis and the neural process.

RESULTS

In one animal (741) which survived for 12½ months, the stalk was found to be intact. The animal, nevertheless, experienced a severe polyuria from the 17th post-operative day, and for 6 weeks the volume of urine excreted each day exceeded

2 l., as compared with a daily average of slightly less than 1 l. during the 2½ weeks before operation. The urinary volume returned to normal after 7 months. The posterior part of the median eminence was found to be damaged, and the neural process partly atrophic. The function of the pars distalis, as judged by the continuance of menstrual cycles, was apparently normal, and no histological evidence of damage could be found.

In another monkey (576) the stalk was also little damaged. This animal, which survived 8 months, experienced a severe polyuria from the 12th post-operative day. This persisted with some diminution until her death. The Polythene had indented and distorted the upper stalk and median eminence from the posterior aspect. The pars distalis was not obviously affected, but the neural process was more cellular than normal.

In a third monkey (744), it proved difficult to assess the degree of success of the operation. The stalk had been severely damaged and, except anteriorly, the Polythene plate, which was rolled up on itself to form a multi-layered structure, intervened between the sella and the floor of the brain. A narrow band of tissue was stretched over the rounded anterior aspect of the Polythene (Pl. 1) and connected the lower part of the pituitary stalk to the brain. Vessels of capillary size were present in this attenuated band of tissue and, theoretically at any rate, constituted a way in which blood could have passed between structures above and below the Polythene. On the other hand, the median eminence was grossly distorted, and only traces of its normal vascular pattern persisted; this was in a region lying above the caudal half of the Polythene. Considerable haemodynamic disturbance must have occurred in the area, and it seems unlikely that any appreciable amount of blood could have flowed directly from the median eminence into the pars distalis along the few small vessels of the stalk remnant. The pars distalis showed no scarring or degeneration, but the neural process was atrophic.

Section of the stalk appeared to have been complete in monkey 625, but it was difficult to be certain that a small part had not escaped. Serial sections through the pituitary region showed that the Polythene had not retained its position between the cut ends of the stalk, and that it lay more caudally in a space containing some amorphous debris, probably indicative of post-operative bleeding into the area. The stalk had been divided in its lower part, and the cut ends were reunited by scar tissue. A fibrous scar was present in the anterior half of the pars distalis (Pl. 2). It measured approximately $1.5 \times 1.8 \times 0.5$ mm., and was most extensive in the coronal plane. This scar extended to the surface of the lobe at its antero-superior pole, but otherwise was separated from the anterior surface by some 0.3 mm. of unscarred glandular tissue. The neural process was much smaller than normal.

Two months after operation this animal showed signs of myxoedema, which responded to treatment with thyroxine. Death occurred 5 months after operation.

In the three remaining animals, section of the stalk was complete and the neural process atrophic. Two of these (nos. 739 and 743) showed scarring in the anterior part of the pars distalis. In 739 the median eminence was normal. The site of transection was in the lower part of the stalk and the upper segment of the stalk ended in a disorganized mass of scar tissue on the superior surface of the Polythene. A prolongation of this tissue extended over the anterior edge of the Polythene, and

contained a few vessels of capillary size which communicated with vessels in the scar tissue below the Polythene. There was a relatively large fibrous zone in the pars distalis, approximately 3.5 mm. wide, 1.5 mm. thick, and 0.5 mm. from back to front. It reached almost to the lateral edges of the pars distalis, and at several points extended to the anterior surface. Numbers of round cystic areas which contained deeply staining amorphous material were associated with it.

In the second of these two animals (743), the cut edges of the stalk had been rejoined by scar tissue. The upper stalk remnant could be clearly defined (Pl. 3), and the floor of the hypothalamus was distorted by the Polythene plate. Vascular channels had reformed along the stalk, and the vessels above and below the plane of section appeared to communicate through the disorganized scar tissue. In spite of this, the pars distalis was shrunken and a large fibrous scar was present in its anterior half, reaching the surface of the gland in one area, and extending about 3.5 mm. from side to side. Even in thick sections it was apparent that the glandular tissue lateral to the scar was less cellular and more fibrous than normal.

The stalk was completely divided in the remaining animal (655). The Polythene plate was well placed and lay immediately below the median eminence, which was flattened and unrecognizable as such. In this case the lower part of the cut stalk was clearly defined (Pl. 4). It extended to the undersurface of the Polythene where it ended in scar tissue, but at a higher level than in the previous three animals. There was no vascular communication between the base of the brain and the pituitary gland.

No scarring of the pars distalis was evident in this specimen, although there were signs that its function was impaired. Thus, menstrual cycles did not continue beyond one post-operative cycle. In spite of distortion of the median eminence and involution of the neural process only a minor degree of polyuria developed.

Vascular anastomoses

In each of the seven animals of this series, the pars intermedia was relatively avascular, while both the neural process and the pars distalis were richly vascularized. Communications across the pars intermedia joined the vascular bed of the neural process to the sinusoids of the pars distalis. These connexions consisted either of single vessels, which reached the size of the larger sinusoids of the pars distalis, or of groups of capillary-size vessels. The pars intermedia was often narrowed in places where numbers of these small vessels crossed.

Vascular communications of this kind were present in every monkey, irrespective of whether section of the stalk had been successful or not, or whether scarring of the pars distalis had occurred. A typical example of a group of small vessels crossing the pars intermedia is illustrated in Pl. 3. In this case the injection of the sinusoids of the pars distalis with indian ink was incomplete, although the vessels of the neural process were well filled. The injection had, however, reached some areas of the pars distalis, particularly tissue lying anteriorly, and tissue adjacent to the pars intermedia. Serial sections show that the sinusoids in the posterior zone of the pars distalis had been filled from the vessels of the pars nervosa, and not *via* the sinusoids of the anterior part of the pars distalis. It thus appears that in each of these monkeys there was a path by which posterior lobe blood, from the inferior hypophysial arteries, could reach the sinusoids of the pars distalis.

DISCUSSION

Scarring of the pars distalis

Section of the stalk was complete in three monkeys of the present series. In two of these (739, 743) the level of section was in the lower part of the stalk, and in both a fibrous scar was present in the pars distalis. In the third animal (655) the level of section was in the upper part of the stalk and the pars distalis was unscarred.

Scarring of the pars distalis was also present in a fourth animal (625). In this monkey the stalk had been greatly damaged and probably divided in its lower part. In a fifth (744) damage to the upper part of the stalk had not resulted in any apparent damage to the pars distalis.

In each case where a scar was present it was situated in the most anterior part of the pars distalis, and reached to the anterior surface at one or more points. Its size varied in different animals, although in all a considerable amount of glandular tissue remained.

Magoun, Fisher & Ranson (1939) have reported that scarring of the pars distalis followed division of the stalk in eight out of twelve rhesus monkeys. On the other hand, Mahoney & Sheehan (1936) found no such changes in a series of twenty rhesus monkeys in which the stalk was clipped (and in three also cut) as far from the tuber cinereum as possible. In the human, Russell (1956) has reported four cases of necrosis of the pars distalis produced either by surgical section of the stalk or by carcinomatous permeation of the portal vessels, and Daniel & Prichard (1958*a*) have recently provided a report of a further case of a like kind. Eckles, Ehni & Kirschbaum (1958), in a short unillustrated abstract, refer to varying degrees of pituitary necrosis in human females after section of the stalk and the insertion of a Polythene plate between the cut ends, and state that in one case at least half the normal amount of pituitary tissue survived. On the other hand, Dandy (1940) has reported a case of mid-stalk section in a girl of 17, in whom no subsequent signs of functional damage to the pars distalis occurred; while Campbell & Harris (1947) noted an area of fibrosis in the pars distalis in only one of forty rabbits in which the stalk was divided.

These differences in the effects of stalk section or stalk damage can hardly be accounted for on the assumption that a variable amount of vascular reconnexion occurs between the cut ends of the stalk (Harris, 1950), since it would be expected that necrotic changes, when they occur, would rapidly follow interference with the blood supply of the tissue concerned. Thus, in the rat, necrosis is present within 24 hr. of section of the stalk (Barnett & Greep, 1951), or cautery of the portal vessels (Daniel & Prichard, 1956).

The more likely explanation is that the degree of damage depends on the level of transection of the stalk, or on anatomical variations in the blood supply to the pars distalis, such as the presence of a direct arterial supply as described in the rabbit (Harris, 1947), and monkey (Wislocki, 1938). In the present series of experiments, section of the lower part of the stalk, or damage to this region, was in each case followed by fibrous scarring of the anterior pituitary, whereas scarring did not follow transection of or damage to the upper part of the stalk. Similarly, Magoun *et al.* (1939) noted that scarring did not occur in their monkeys when section was at

the level of the median eminence. Similar findings have recently been reported in other species. Daniel & Prichard (1957*b*) found extensive necrosis of the pars distalis of the sheep when the animals were killed 1–4 days after section of the lower stalk. If they were allowed to survive 12½–18 weeks greater amounts of healthy pars distalis tissue were found, and the necrotic zone had become transformed into a relatively small fibrous area (Daniel & Prichard, 1958*b*). Similar results have been reported in goats (Daniel & Prichard, 1958*c*). These workers conclude that the initial necrosis is due to interruption of the ‘long’ portal vessels, and that the disposition of the ‘short’ vessels in the lower infundibular stem is such that these are not damaged by the transection, so that the tissue of the pars distalis, which the latter vessels supply, survives. These vessels are supplied by the inferior hypophysial arteries, and the surviving glandular tissue lies immediately adjacent to the ‘lower infundibular stem’.

Vascular communications

Anastomoses across the pars intermedia have been found in the glands of all the monkeys described above. It is, however, impossible to assess the exact functional importance of these channels from vascular injections made after death. On the other hand, since scarring has never been reported in the posterior zone of the pars distalis after stalk section, it would seem that enough blood always passes through these anastomotic vessels, and through ‘short’ portal vessels in the lower part of the stalk, to prevent infarction of that part of the pars distalis which lies adjacent to the pars intermedia. From the fact that no scarring seems to occur in any part of the pars distalis when the stalk is divided close to the median eminence, it also seems to follow that the higher the plane of damage to the stalk, the greater is the possibility that blood can continue to flow, through long portal vessels, to all regions of the gland. Presumably the capillary plexus in most of the stalk is a continuous vascular network which is fed from below as well as from above.

Regeneration

There is a possibility of regeneration of pars distalis tissue in animals which survive section of the stalk for more than a few days. Mitotic activity in the anterior lobe has usually been described as very slight, although Dawson (1942) estimated a total of 39,000 cells in mitosis in the gland of a castrate female monkey treated for some weeks with oestrogen and testosterone, and given colchicine 6½ hr. before death.

Recent work of Daniel & Prichard (1958*b, c*) indicates that regeneration of pars distalis tissue may occur after the acute phase of infarction which results from section of the stalk. They found mitotic activity in the pars distalis of goats within 3 days of operation, and in both sheep and goats the area of fibrosis after a lapse of some weeks was much smaller than the area of the infarct in animals killed within hours or days of operation.

The scarred anterior lobes of the monkey pituitary glands described above resemble those in the published illustrations of Daniel & Prichard. It may be concluded that fibrosis of the infarct, and later contraction of the scarred area, had followed the acute phase of necrosis in these animals. Regeneration of pars distalis might also have occurred, but there was no definite evidence for this. In spite of the

fact that 5–7 months had elapsed since operation, relatively large scars were still present, and it seems unlikely that these would disappear even after longer periods of post-operative survival.

SUMMARY

1. Section of the pituitary stalk was attempted on eight female rhesus monkeys.
2. Partial or complete transection of the lower but not the upper part of the stalk was followed by fibrous scarring in the anterior pars distalis.
3. Vascular connexions between the neural process and the pars distalis were found in all the animals.
4. It is considered that blood which passes through the latter vessels, and through 'short' portal vessels, prevents necrosis of the posterior part of the pars distalis after section of the lower stalk.

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EXPLANATION OF PLATES

All figures are photomicrographs of 50 μ sections cut in a sagittal plane. *N*=neural process; *I*=pars intermedia; *PD*=pars distalis; *S*=pituitary stalk; *F*=fibrous scar; *P*=Polythene.

PLATE 1

Monkey 744. The undivided pituitary stalk is stretched over the anterior surface of the Polythene, but it has been damaged in its upper part. The neural process is shrunken. No scarring of the pars distalis has occurred. $\times 25$.

PLATE 2

Monkey 625. Section of the stalk was probably complete at a low level. The neural process is atrophic, and there is a fibrous scar in the anterior half of the pars distalis. $\times 45$.

PLATE 3

Monkey 743. Section of the stalk is complete, but the Polythene has become displaced and scar tissue has filled the gap between the cut ends. The neural process is reduced in size. The pars distalis is shrunken and contains a large scar. A group of small vessels (*V*) runs between the neural process and the pars distalis. $\times 25$.

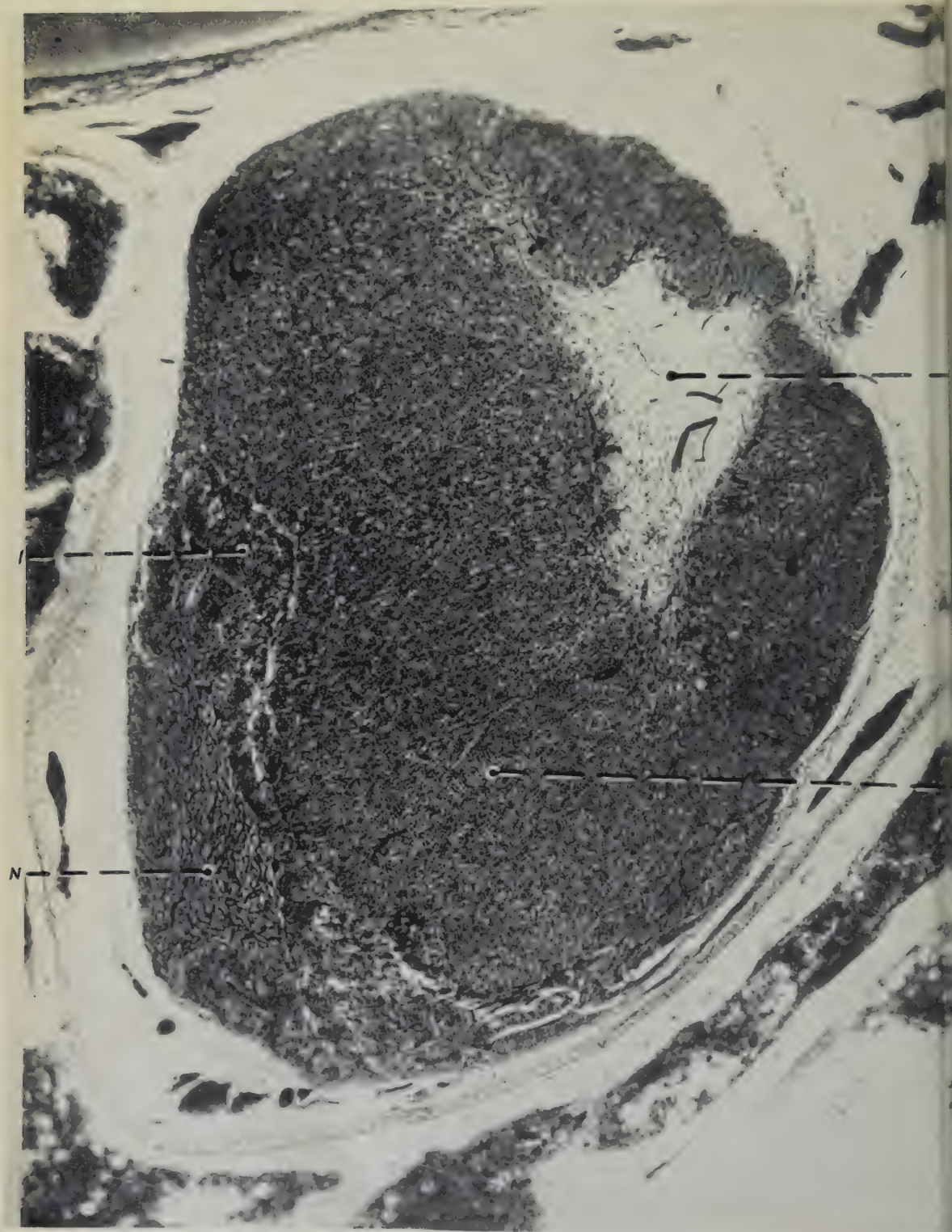
PLATE 4

Monkey 655. The stalk has been divided in its upper part, and the lower part is undamaged. There is no scarring of the pars distalis. $\times 25$.

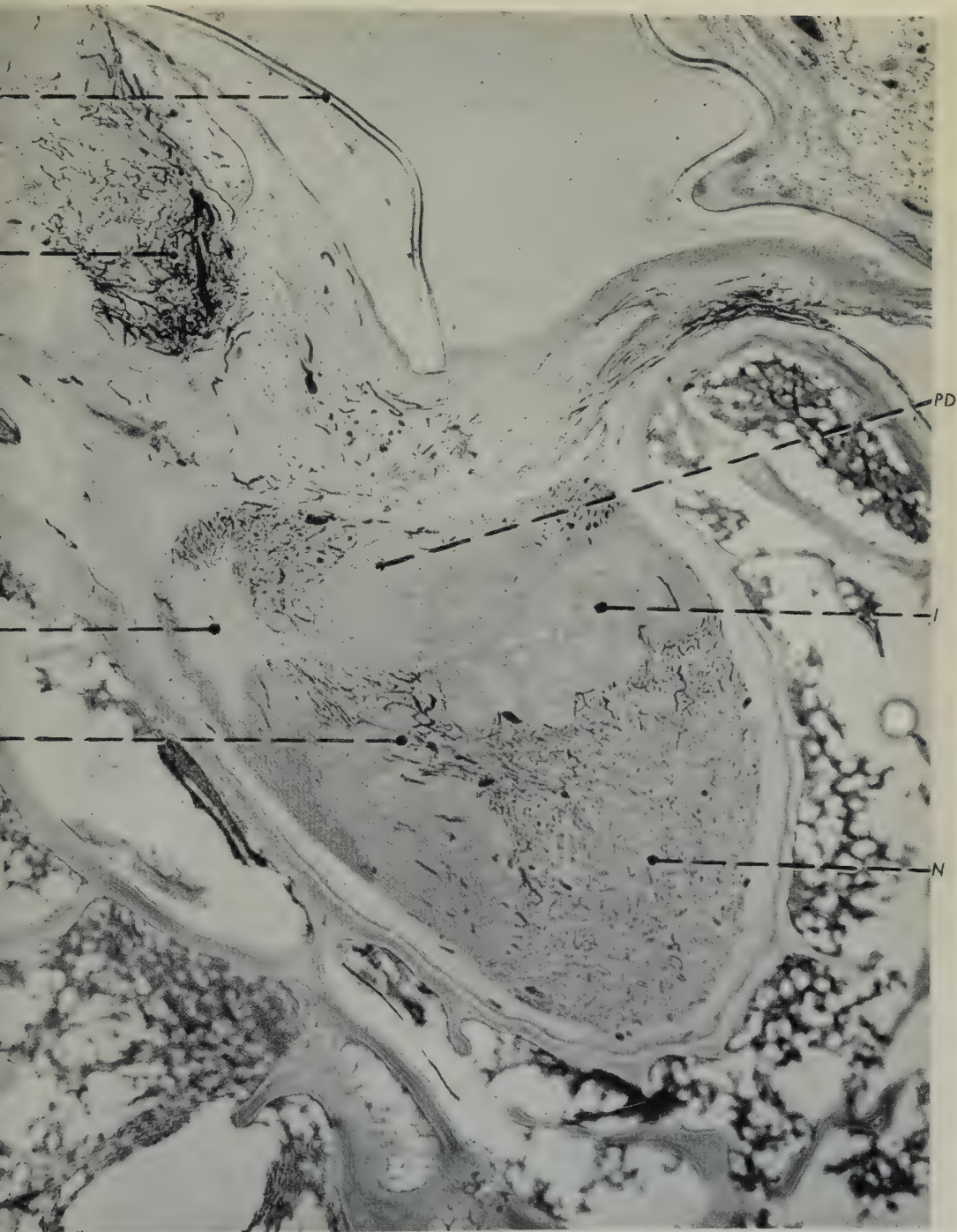


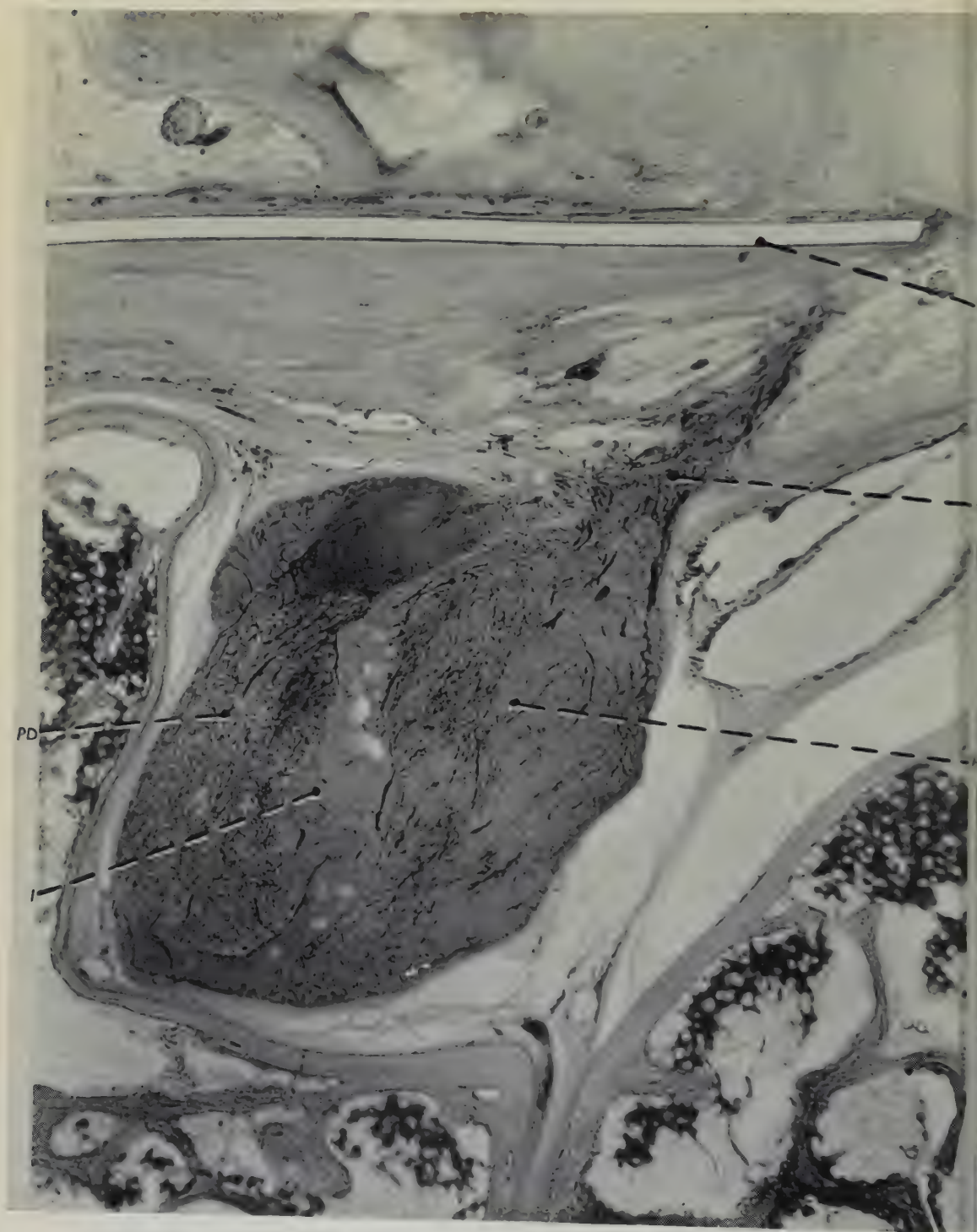
HOLMES AND ZUCKERMAN—THE BLOOD SUPPLY OF THE HYPOPHYSIS IN *MACACA MULATTA*

(Facing p. 8)



HOLMES AND ZUCKERMAN—THE BLOOD SUPPLY OF THE HYPOPHYSIS IN *MACACA MULATTA*





HOLMES AND ZUCKERMAN—THE BLOOD SUPPLY OF THE HYPOPHYSIS IN *MACACA MULATTA*

NUCLEAR MULTIPLICATION AND CELL MIGRATION IN DEGENERATING UNMYELINATED NERVES

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INTRODUCTION

Joseph (1947, 1950) has reported that when an unmyelinated nerve, the anterior mesenteric of the rabbit, underwent degeneration, there was no increase in nuclear population such as occurs so strikingly in myelinated nerves (see Abercrombie & Johnson, 1946; Thomas, 1948). Rexed & Fredriksson (1956), on the other hand, have found mitoses in two different degenerating unmyelinated nerves, the splenic and the thoracic vagus of the guinea-pig. In the present paper we have investigated nuclear population changes in yet another unmyelinated nerve, the abdominal vagus of the rabbit. We have also taken the opportunity to assess the behaviour in tissue culture of explants of this nerve during degeneration, for comparison with earlier results on a myelinated nerve (Abercrombie & Johnson, 1942).

The abdominal vagus of the rabbit, like all so-called unmyelinated nerves, contains a few myelinated fibres (all of less than $6\ \mu$ diameter): an average of 75 amongst about 26,000 unmyelinated (Evans & Murray, 1954*a*). It has the advantages that its fibres can be severed in the neck, so that the operative trauma does not complicate the response of the nerve in the abdomen; and that after such an operation it undergoes substantially no re-innervation, the axons emerging from the central stump being diverted into the recurrent laryngeal and other branches of the vagus containing myelinated fibres (Evans & Murray, 1954*b*), so that the effects of re-innervation on outgrowth in tissue culture (Abercrombie, Johnson & Thomas, 1949) are avoided.

MATERIAL AND METHOD

The investigations were made on the abdominal vagus nerves of adult rabbits. The nerves enter the abdomen as two main trunks placed on the dorsal and ventral surface of the oesophagus. Microscopically at this level the nerves are composed almost entirely of closely packed unmyelinated fibres, the great majority of the myelinated fibres present in the cervical vagus having been distributed in the recurrent laryngeal, cardiac, bronchial and oesophageal branches (Evans & Murray, 1954*a*).

Degeneration was produced by cutting the left cervical vagus at the level of the thyroid cartilage, using Nembutal and ether anaesthesia and aseptic precautions. The two cut ends were separated by a gap of 2–3 cm. This results in degeneration of about half the total number of abdominal vagus fibres, the ventral branch containing more of the degenerated fibres than the dorsal (Evans & Murray, 1954*a*). After survival periods of 5, 10, 25 or 100 days the animals were killed with an overdose of Nembutal. All of the macroscopically unbranched dorsal and ventral vagus nerves below the diaphragm (usually about 1.5 cm. of each) were removed and each cut

into two lengths. The proximal half of the ventral branch was taken for tissue culture and both distal halves were fixed in Bouin's fluid. The fixed nerves were embedded in paraffin, sectioned transversely (some also longitudinally) at $5\ \mu$, and stained with haematoxylin and eosin. Nuclear counts were made at a magnification of 1200 diameters. In this study we have made only total nuclear counts, combining those of the dorsal and ventral branches. It was impossible to make reliable differential counts of the various cell types. By taking the number of nuclei in the whole cross-section of nerve rather than in a unit area of section it is possible to ignore volume changes due to oedema in the nerve produced by degeneration. Nuclear lengths were measured in two nerves of each period of degeneration, sectioned longitudinally.

In the tissue culture experiments, explants of the whole thickness of the ventral branch of the nerve were placed in hanging drops on coverslips. The medium consisted of fowl plasma, extract of 8-day-old chick embryos and Pannett and Compton's saline. Four explants from the same piece of nerve were placed in each clot. Nerves of all the different degeneration times were cultured simultaneously. After 3 days at 38°C ., the cultures were fixed in formal-saline, stained in alum haematoxylin, mounted and examined.

RESULTS

Nuclear population. The mean number of nuclei in one transverse section of the whole abdominal vagus (both branches combined) at different times of degeneration is given in Table 1. Though the nuclear volume probably increased (compare Pl. 1, figs. 1, 2), the mean nuclear dimension in the long axis of the nerve, given in Table 1, did not change appreciably during degeneration; the counts have therefore not been standardized to eliminate the section error (Abercrombie & Johnson, 1946).

Table 1. *Nuclear population during degeneration*

(The mean number of nuclei of all kinds seen in a transverse section of the nerve is given with its standard error. The last column gives the mean nuclear length normal to the plane of transverse section in a sample of nerves.)

Days of degeneration	No. of animals	Mean nuclear population	Nuclear length (μ)
0	6	632 ± 39	12.9
5	7	1013 ± 68	13.0
10	5	1024 ± 48	13.1
25	6	778 ± 88	12.9
100	4	605 ± 68	12.6

The increase of nuclear population during the first 5 days of degeneration, amounting to 60 %, is highly significant ($t\ 4.65$, D.F. 11, $P\ 0.001$). There is no change between 5 and 10 days but thereafter the population falls. It is already significantly less at 25 days ($t\ 2.31$, D.F. 9, $0.05 > P > 0.02$). Neither the mean at 25 days nor at 100 days is significantly different from the mean of the normal nerves.

Histologically the main change in the cell population during degeneration is the appearance, particularly at 5 and 10 days, of large numbers of rounded nuclei (Pl. 1, fig. 2) belonging to macrophage-like or sometimes lymphocyte-like cells.

Rarely a polymorph was observed in the endoneurium of 5-day nerves. In the longitudinal sections counts were made of the relative proportions of rounded and of elongated nuclei, amongst a total of about 200 cells in each nerve. In the normal nerves the rounded nuclei amounted to about 2 % of all nuclei, at 5 days 19 %, at 10 days 15 %, at 25 days 11 %, and at 100 days 5 %. There are not therefore enough of such cells to account for the entire population increase, nor conversely for the decrease after 10 days. The presence of these rounded nuclei does not alter the mean length of all nuclei because of a concomitant increase in the proportion of larger nuclei. Mitoses were seen in the 5- and 10-day nerves. In one 5-day nerve a differential count of fifty-two mitoses (0.4 % of all nuclei present) was made. Eleven mitoses were identified in Schwann cells: four of these were obviously within the tubes of degenerating myelinated fibres, and seven were judged to belong to unmyelinated fibres because of their position in the midst of strands of such fibres and their frequent elongated shape (Pl. 1, fig. 4). Twenty-seven mitoses were clearly in the endoneural spaces, made obvious by the oedema: fifteen of these were probably of macrophages, six especially large mitoses with abundant structureless cytoplasm were judged to be fibrocytes, one was in vessel endothelium and five could not be allocated. It was not certain whether the remaining fourteen mitoses were in the endoneurium or were of Schwann cells. A few pycnotic nuclei were seen in most nerves, but not especially frequently in the nerves degenerated 25 days, which might be expected to be undergoing a diminution of cell population.

Outwandering in vitro. During cultivation cells emigrate after a latent period of 1-2 days from the cut ends of each nerve fragment. Counting of cells was done after fixation on the third day of culture. Only cells whose nuclei had emerged were recorded. No allowance has been made for mitosis amongst cells that have already migrated into the medium. Schwann cells (Pl. 1, fig. 3) were identified as follows: they are bipolar, consisting basically of a long fine unbranched smoothly contoured thread with a swelling containing the nucleus at some point on its length; they commonly associate end to end without any visible limit between one cell and the next; and when numerous they associate, less intimately, side by side. Fibroblasts were placed in two categories, those similar to Schwann cells in that they were much elongated but distinguished by branching processes and relatively more cytoplasm especially around the nucleus; and those of conventional fibroblast form. No sharp distinction between these two kinds of fibroblast could be made, because of intermediate forms, and the counts of the two categories at the different degeneration times were highly correlated with each other, so they have been grouped together for tabulation.

The mean numbers of nucleated cells found outside each explant after 3 days *in vitro* is given for the different times of degeneration in Table 2. Half of the fifty explants of undegenerated nerve produced no cells at all. Only six of them produced Schwann cells, though many had numerous fibrillar cytoplasmic processes projecting from the cut ends of the nerve which may have been parts of Schwann cells. After 5 days of degeneration, and subsequently, behaviour *in vitro* was quite different. Very large numbers of macrophages appeared (Pl. 1, fig. 3) and some fibroblasts (the two categories of fibroblast in roughly equal numbers). Schwann cells were also present, mostly as single cells.

Beyond 5 days of degeneration there was a steady decline in the numbers of fibroblasts and macrophages in the outgrowth. There is a suggestion, however, that the Schwann cells emerge more actively at 10 days than at 5 days. Though the difference between these two times of degeneration in numbers of Schwann cells is not significant the ratio of Schwann cells to fibroblasts does change significantly. At 5 days this ratio is 0.25 ± 0.13 and at 10 days 1.10 ± 0.20 ; for the difference t is 3.63, D.F. 8, P 0.01. The outwandering activity of Schwann cells has therefore a different time-course from that of fibroblasts. Many separate strands of Schwann cell cytoplasm emerge from the cut ends of the 10-day nerves. It is clear from their large number and their distribution that they cannot be coming only from the myelinated fibres of the nerve. At 25 days there is a general diminution of the amount of outwandering. At 100 days three of the nerves were entirely devoid of emigrated cells, apart from fine cytoplasmic fibrils. Three others were more active than any of the normal nerves and resembled those of the 25-day group.

Table 2. *Numbers of cells emigrated per explant, after 3 days in culture, of nerves degenerated for different periods*

Days of degeneration	No. of animals	Schwann cells	Fibroblasts	Macrophages
0	6	0.1 ± 0.1	5 ± 3	6 ± 4
5	5	19 ± 6	150 ± 49	530 ± 90
10	5	39 ± 7	45 ± 8	220 ± 66
25	4	13 ± 4	25 ± 6	80 ± 20
100	6	7 ± 4	14 ± 9	22 ± 12

DISCUSSION

The increase of nuclear population during degeneration, reported here in the abdominal vagus of the rabbit, agrees with the results of Rexed & Fredriksson (1956) on the thoracic vagus of the guinea-pig, rather than with those of Joseph (1947, 1950) on the anterior mesenteric of the rabbit. The increase is at least in part the result of mitoses, which, as is usual in such proliferations (Abercrombie, 1957) probably occurred in all types of cell. A contribution to the increased population by invasion from the blood vessels cannot however be ruled out. Many rounded mononuclear cells are present in the endoneurium, especially after 5 days of degeneration, with here and there a granulocyte. The mononuclears appear to be mostly macrophages and this is confirmed by the emigration in tissue culture, the wandering cells of which are overwhelmingly macrophages. The postulation of an invasion of blood-borne cells is not however necessary. The macrophage infiltration is also explicable as the result of the rounding up of histiocytes in the oedematous endoneurium, together with their mitosis: many of the mitoses observed appeared to be of macrophages. The concentration of macrophages after 5 days of degeneration, observed both in sections and in cultures, in our experience much exceeds that found during degeneration of myelinated nerves.

The amount of nuclear increase is small, but it must be remembered that only 50 % of the fibres were degenerated. The increase seems roughly comparable with that which would result from the numbers of mitoses recorded by Rexed & Fredriksson.

Recent work on myelinated nerves (Abercrombie & Santler, 1957) has suggested that the correlation previously found of amount of nuclear population increase with mean fibre diameter (Thomas, 1948; Joseph, 1950) is not the important relationship in the proliferation of degenerating nerves; but rather that the total mass of nerve fibre destroyed within the nerve and the initial density of cell population are what determine the amount of multiplication. Nerve fibre mass has a positive, initial density an inverse relation to multiplication. The volume of nerve fibre in the abdominal vagus is unknown, but the initial density of population is very high, so it is to be expected that the nuclear population would grow by a small factor. The earlier time at which the maximum nuclear population is reached, probably at 5 days or soon after, agrees with Thomas's observations (1948) that the smaller the average fibre diameter the earlier is the population peak.

The decrease of population which probably sets in between 10 and 25 days is different from the decline occurring in myelinated nerves in that the population returns substantially to that of a normal nerve, instead of remaining somewhat elevated. The mechanism of the cell loss during the later stages of nerve degeneration is unknown, so no hypothesis can usefully be suggested about the peculiarity of the unmyelinated nerve. The diminution of the number of rounded macrophages, though it is considerable, is not enough to account for all the cell loss.

The tissue culture method tests cell movement rather than mitosis. The rise and fall of migratory activity with the progress of degeneration occurs much as in myelinated nerves (Abercrombie & Johnson, 1942), in that it is roughly similar to, but much more extreme than, the rise and fall of nuclear population. The wave of migratory activity is probably in part a reflexion of the stimulation that produces the wave of mitosis (Abercrombie, 1957); and correspondingly both waves are passed through more quickly in the unmyelinated nerve. It has been possible to show in the vagus that the increased Schwann cell emigration during degeneration is delayed in relation to the increased fibroblast emigration, a dissociation which has not yet been investigated in myelinated nerve. Its explanation may be the restraint on Schwann cell movement exerted by axons, probably through mutual adhesion (Abercrombie *et al.* 1949), which gradually decays as the axons break up.

SUMMARY

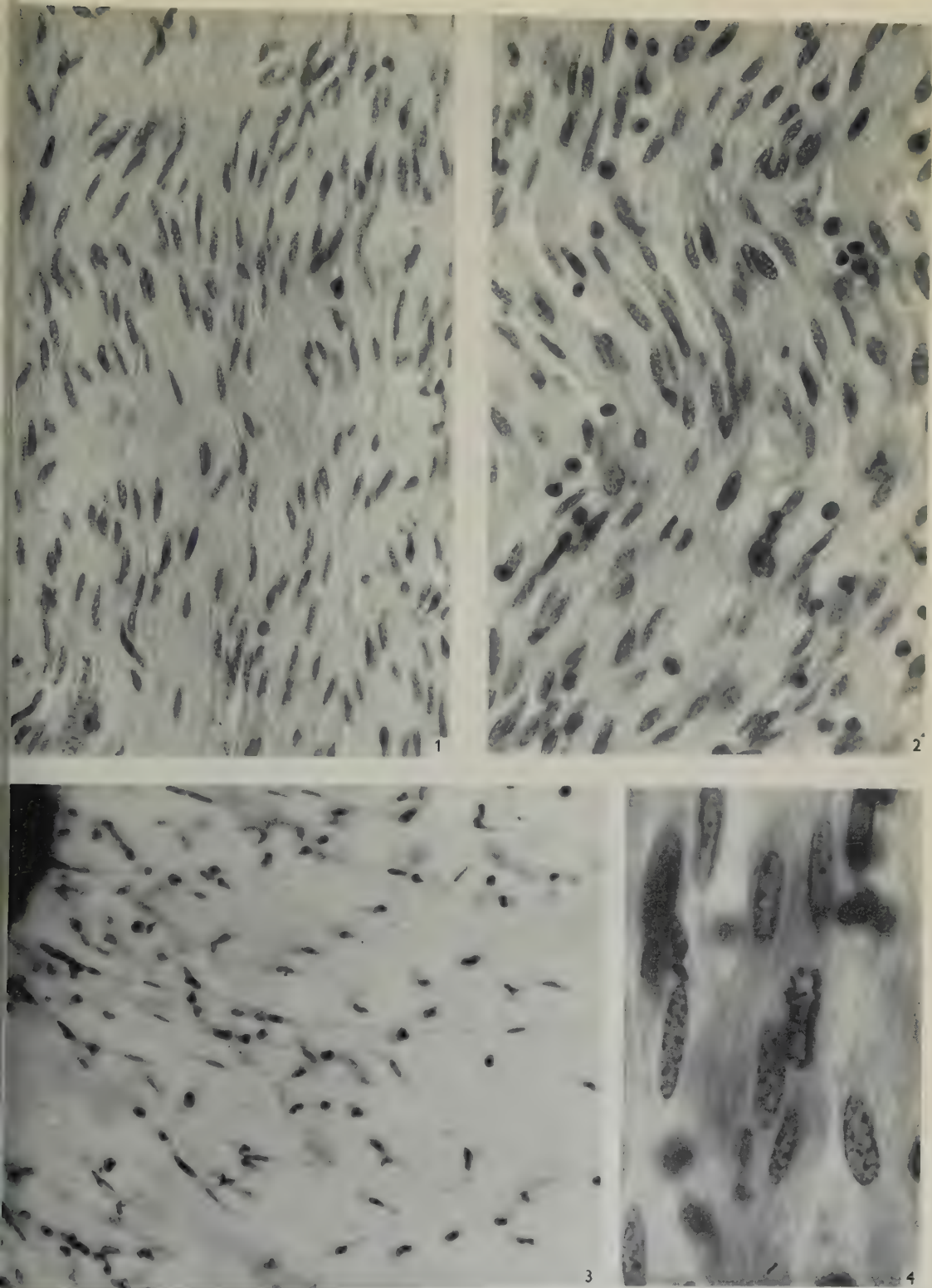
Degeneration of nerve fibres was produced in the unmyelinated abdominal branches of the vagus nerve in rabbits by severing one vagus nerve in the neck. A significant increase from the normal in total nuclear population of the degenerated nerve was found at 5 and 10 days after the lesion. Mitoses were observed, probably in all types of cells. After 10 days the population declined, and by 100 days had returned to normal. Explantation of fragments of the nerves showed a great increase in emigration of Schwann cells, fibroblasts and macrophages, as compared with the normal nerves, during the early phases of degeneration, returning to near normal at 100 days of degeneration. The maximum activation of Schwann cell outgrowth was probably a little delayed in relation to the maximum activation of fibroblast and macrophage outgrowth.

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EXPLANATION OF PLATE

- Fig. 1. Normal (undegenerated) abdominal vagus nerve in longitudinal section. $\times 500$.
- Fig. 2. Abdominal vagus nerve degenerated for 5 days. The nuclei are swollen, though on the average no longer than in normal nerve, and a number of round cells are present. $\times 500$.
- Fig. 3. Tissue culture, fixed and stained after 3 days *in vitro*, of vagus nerve previously degenerated for 10 days. Schwann cells and numerous macrophages. $\times 200$.
- Fig. 4. Prophase presumed to be of a Schwann cell of an unmyelinated fibre because of its situation and elongated form. $\times 1200$.





THE VASCULAR ANATOMY OF THE MEDIAN NERVE IN THE FOREARM AND HAND

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Available accounts of the gross arterial blood supply of the median nerve, such as those furnished by Quénu & Lejars (1892); Bartholdy (1897); Bourguet (1913); Ramage (1927); Dieulafoy (1931) and Sunderland (1945*a*) exhibit considerable diversity of statement, and, so far as can be ascertained, the venous drainage of the nerve and its intrinsic vascular anatomy have not been described. Because of these considerations and in view of the possible relevance of median nerve vasculature to the aetiology of the carpal tunnel syndrome, it was decided to reinvestigate the gross blood supply of the median nerve in the forearm and hand and to study its intrinsic vascular arrangements.

MATERIAL AND METHODS

The investigation was carried out on ten adult upper limbs amputated through the upper arm: five of these were injected via the brachial artery with coloured neoprene latex, one was injected with a suspension of 5% indian ink in double-strength plasma, and one with monastral fast blue BNVS paste. The neoprene and indian ink injected specimens were dissected to display the gross disposition of the blood supply of the median nerve; the specimen injected with monastral fast blue was used to study the intrinsic vascular anatomy, the nerve being removed from the forearm and hand and cut into segments which were sectioned, alternately, longitudinally or transversely, at 200 or 400 μ : some of the sections were counterstained with eosin.

The nerves from two of the uninjected limbs were similarly removed and cut into segments, which were then treated by a modified Pickworth technique (Steele & Blunt, 1956), both longitudinal and transverse sections being cut at 200 μ . Segments of the nerve from the remaining uninjected limb were embedded in paraffin, and 25 μ transverse sections were cut at various levels and stained with haematoxylin and Biebrich scarlet.

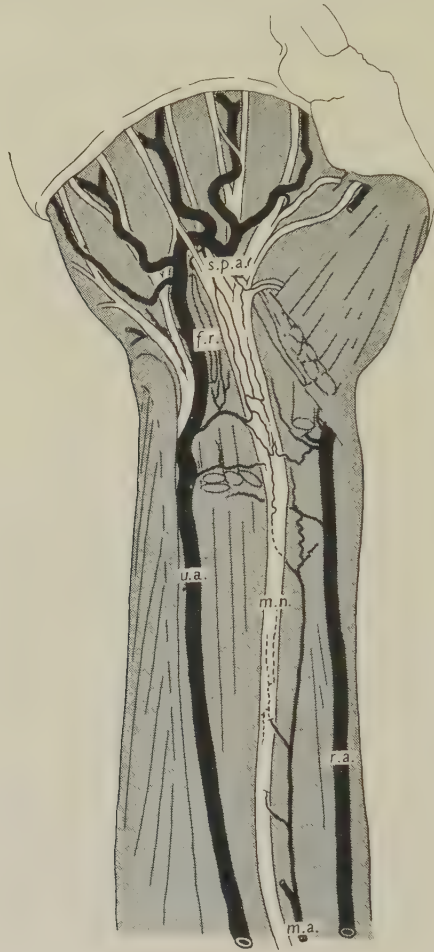
RESULTS

The gross blood supply

In each of the six limbs dissected to show the gross features of its vascularization, the median nerve below the elbow was supplied by the superficial palmar arch, by the ulnar artery just proximal to the flexor retinaculum, and by the median artery more proximally in the forearm (Text-fig. 1).

The branch from the superficial palmar arch was found, in each instance, to course transversely just distal to the terminal gangliform enlargement of the median nerve trunk, to give off variable branches to the digital nerves and to continue on to the

nerve to the thenar muscles. From the proximal aspect of this vessel two or three ascending branches were found to ramify in the epineurium, both on the anterior aspect of the nerve trunk and in its interfascicular connective tissue, and to ascend to anastomose with more proximal neural nutrient arteries (Text-fig. 1). In four of the six limbs the same transverse vessel gave a similar branch which ascended on



Text-fig. 1. Drawing from a dissection of a neoprene injected limb to show the arterial blood supply of the median nerve.

the posterior aspect of the nerve and this, besides providing epineurial and intra-neural branches, gave off extraneural branches which ramified on the anterior surface of the common synovial flexor sheath. In the remaining two instances this posterior ascending branch was derived separately from the superficial palmar branch of the radial artery.

Characteristic of the blood supply of the median nerve was the occurrence of a branch of the ulnar artery which reached it just proximal to the flexor retinaculum (Text-fig. 1). Before gaining the nerve this vessel invariably gave branches to the

anterior surface of the common synovial flexor sheath: it was found to divide terminally into ascending and descending branches which anastomosed, in the epineurium on the anterior surface of the nerve, with adjacent epineurial vessels derived from the superficial palmar arch on the one hand and from the median artery or the radial artery on the other.

In none of the six injected limbs was the median artery sufficiently well developed to gain the wrist, and in two of them it was extremely small, running but a very short course in the upper third of the forearm close to the median nerve. The vessel derived in three specimens from the common interosseous artery, in two from the ulnar artery, and, in the remaining limb, from the anterior interosseous artery. It was found to give from one to three nutrient arteries to the median nerve and was largely expended in muscular branches, its final termination being muscular or neural with equal frequency. Neither the size nor the number of the neural nutrient branches of the median artery appeared to be directly related to the size of the parent vessel. In one limb, wherein the median artery was so short as to render difficult its consideration as a true median artery rather than a mere muscular twig of the ulnar artery, it provided two nutrient branches to the nerve; whilst in another specimen, wherein the median artery accompanied the median nerve to within $1\frac{1}{2}$ in. of the flexor retinaculum it provided but a single nutrient neural branch, and that no larger than the homologous branch given off by the contrastingly shorter median artery.

The nutrient neural arteries described above were of constant occurrence in the present series, but additionally the median nerve receives an arterial supply from other, less constant, sources. In the distal forearm neural branches of the radial artery, or of its anterior carpal branch, were encountered in all the present specimens save one. From one or both of these sources three specimens exhibited two neural nutrient vessels, two specimens showed one nutrient neural vessel (from the radial artery) while one specimen showed no such supply. The nutrient neural branches of the radial and anterior carpal arteries also give twigs to the common synovial flexor sheath and may (as in two of the present specimens) anastomose directly on the anterior surface thereof with twigs from the corresponding nutrient neural branch of the ulnar artery.

The median nerve may receive a nutrient artery in the cubital fossa. In four of the six limbs specially dissected this artery came from the anterior ulnar recurrent artery. In the remaining two specimens in place of such a cubital artery the median nerve was vascularized at a slightly higher level, in one instance by the brachial artery, in the other by its supratrochlear branch. In one specimen an additional neural nutrient branch from the ulnar artery joined the nerve in the distal forearm (Text-fig. 1).

The pattern of venous drainage external to the nerve trunk was found, in all cases, to conform closely to that of the arterial blood supply. Small veins accompanying each of the neural nutrient branches of the superficial palmar arch and the radial and ulnar arteries drain into the *venae comitantes* of those main arterial trunks: the veins following the neural nutrient branches of the median artery drain into a small vein which accompanies the latter vessel.

INTRANEURAL VASCULAR ARRANGEMENTS

Throughout its extent in the forearm the median nerve is well fasciculated, its fasciculation increasing markedly, as Sunderland (1945*b*) has noted, in approximately its distal 3 in. A surface epineurial vascular plexus and inter- and intrafascicular vascular plexuses, all in communication with one another, are demonstrable throughout the course of the nerve in the forearm and hand (Pl. 1, figs. 3, 4). The vessels constituting these plexuses run, for the most part, longitudinally, and are linked to one another by both oblique and transversely running connexions so as to form a free vascular network. In the surface epineurium and in the interfascicular connective tissue thick-walled vessels of arteriolar or larger size are constantly encountered (Pl. 1, figs. 1-4), and not uncommonly arterioles and venules are to be found in the perineurium. Within fasciculi, however, except occasionally in association with perineurial septa, vessels with walls recognizably muscular are not encountered. The surface epineurial vascular plexus is distinct from the deeper lying perifascicular plexus in that it is particularly rich in capillary vessels, and these are fed and drained not only by the surface arteries and veins in the epineurium, but also by the interfascicular arteries and veins (Pl. 1, fig. 4): the latter vessels also supply the capillaries of the perifascicular and intrafascicular plexuses. An identical arrangement has been noted in sections cut from the injected antebrachial ulnar nerve.

Distal to the flexor retinaculum there is a marked increase in the amount of interfascicular connective tissue, which here becomes secondarily condensed around bundles of fasciculi and contains arteries and veins of particularly large relative size (Pl. 1, fig. 2). Several arteries, of a size equal to that of the extraneural parts of many neural nutrient arteries, are to be found running longitudinally between the bundles of fasciculi, and these ascend to effect direct anastomotic connexions with the regional nutrient vessels proximal to the flexor retinaculum. Both inter- and intrafascicular vessels are continued longitudinally into the terminal branches of the median nerve, wherein their arrangement frequently becomes more irregular than elsewhere in presumptive correlation with the plexiform fascicular branching.

DISCUSSION

Present findings concerning the gross arterial supply of the median nerve are in close agreement with those of Bartholdy (1897) and Dieulafé (1931), and agree in general with those of Tonkoff (1898). They differ from the observations of Quénu & Lejars (1892) in that these authors described no neural nutrient branch from the ulnar artery: they did not comment on their failure to find this vessel (already noted by von Haller, 1758), nor did they give any indication of the number of dissections forming the basis of their specific observations.

The blood supply of the median nerve has also been studied by Bourguet (1913), and observations on its vascularization in the forearm, but not in the hand, have been added by Ramage (1927) and Sunderland (1945*a*). Bourguet stated that the median nerve in the forearm is supplied solely by a branch of the ulnar artery (presumably the median artery), from which a series of nutrient vessels arise, each of which has a limited area of distribution within the nerve. Tonkoff had stated

earlier that occasionally, and when well developed, the median artery may exclusively supply the antebrachial portion of the nerve, an arrangement encountered by Ramage in one of twenty-three dissections. It appears possible, therefore, that Bourguet, who did not detail the material of his investigation, made his observations on a single variant specimen. In the present investigation no appreciably large median artery was encountered, but, within the limits of the variations observed, no evidence was forthcoming to suggest that the extent to which the median artery dominates the blood supply of the nerve is dependent upon its degree of development. Unfortunately, neither Tonkoff nor Ramage indicate the size or distribution of the median artery in those instances wherein it was found to supply the nerve exclusively. The possibility that an unusually large median artery may sometimes be directly associated with the occurrence of neurovascular lesions cannot be overlooked, but this seems to have escaped comment in the relevant literature.

Ramage's observations on the vascularization of the antebrachial median nerve were based upon the examination of eight adult and fifteen foetal upper limbs. Regarding the main sources of arterial supply his findings are conformable with those of the present investigation, though indicative of a more variable vascular pattern, particularly in connexion with the adult limb. Ramage found, for example, that the median and anterior interosseous arteries together supplied the nerve in all the foetal limbs studied but in only five of the adult limbs, while direct or indirect neural nutrient branches of the ulnar artery were found to supply the nerve in only three adult and eight foetal specimens. However, since his injections were made into the aorta or the subclavian artery, it seems very probable that vascular filling was sometimes incomplete in the distal parts of the limbs. On the basis of the present findings it is concluded that the contention of Quénu & Lejars, that the blood supply of peripheral nerve trunks is constant, whilst not entirely valid for the median nerve, has sufficient value to recommend it as a useful working guide.

Sunderland (1945*a*) claimed that the median artery, when present, constituted the main arterial blood supply of the median nerve in the forearm, and that this vessel and its branches to the nerve constitute a 'single nutrient system'. However, the findings of Bartholdy, Tonkoff and Ramage, together with those of the present investigation support Tonkoff's interpretation of the median artery as an *arteria comitans*, that is, as a vessel chiefly concerned with the supply of structures other than the median nerve, rather than as a true *arteria nutritia*. In substantiation of his claim that in three out of thirty-seven specimens the median nerve received its last nutrient artery in the cubital fossa, Sunderland does not appear to have extended his search for neural nutrient vessels into the palm, and in view of the reports of Quénu & Lejars, Bartholdy, Tonkoff and Dieulafé, supported by the present findings, that the median nerve is invariably supplied by the superficial palmar arch, this claim is unconvincing. Moreover, in the light of the results of the many and varied investigations of the blood supply of nerves in general, it appears unlikely that a peripheral nerve trunk should run so long a course as that of the antebrachial median nerve without any regional supply. In twelve of Sunderland's specimens the median nerve was stated to be supplied by vessels of unascertained origin, and the relevant dissections were made on material apparently not specifically injected for the purpose of demonstrating the finer blood vessels. For these reasons, and in

view of the considerable difficulty of recognizing vasa nervorum (other than those containing blood) unless filled by the use of a suitable injection material, Sunderland's results cannot be accepted uncritically.

Bichat's (1824) contention that the veins of nerves, like cerebral veins, take courses independent of those of the corresponding neural nutrient arteries is not confirmed for the median nerve. Present findings are in harmony with the general principles governing the venous drainage of peripheral nerve trunks as enunciated by Quénu & Lejars.

The observations of Ranvier (1878) on the intrinsic vascular anatomical pattern of nerve trunks are exemplified for the median nerve by the present study. The additional observation is made, however, that the rich surface epineurial capillary plexus is distinct from the perifascicular capillary plexus, and is fed and drained, on the one hand, by the arteries and veins of the surface epineurium, and, on the other, by the underlying interfascicular vessels. This arrangement is not peculiar to the median nerve, for it has been demonstrated also in the antebrachial ulnar nerve. It is concluded that the deep venous drainage of the surface epineurium may serve to prevent the venous engorgement otherwise resulting from a relatively minor degree of external compression of a segment of nerve trunk. The characteristic findings of relatively large arteries in the interfascicular connective tissue at the distal end of the median nerve appears of interest since Denny-Brown & Brenner (1944) have shown that the 'compartmented structure' of a peripheral nerve trunk may serve to protect intraneural vessels from the effects of external compression. The architecture of the median nerve in approximately its distal 3 in. is extremely fascicular, and expectedly these large interfascicular vessels would be highly resistant to the effects of external compression. It is relevant, therefore, to note that although external compression of the median nerve under the flexor retinaculum has been frequently invoked as the cause of the carpal tunnel syndrome, operative findings have never provided convincing evidence that such compression regularly exists. Garland, Bradshaw & Clark (1957), for example, noted that in approximately two-thirds of fifty-three operative cases abnormal findings of any sort were plainly wanting.

Whilst there is evidence to show that the regional blood supply of peripheral nerve trunks is functionally segmental (Blunt & Stratton, 1956), evidence for structural segregation of adjacent neural nutrient arteries, such as claimed by Bourguet to exist in the median nerve, has not been encountered in the material under present investigation. In the median nerve, as in other peripheral nerve trunks, adjacent regional nutrient arteries are freely and mutually linked by longitudinal anastomoses both in the surface epineurium and in the interfascicular connective tissue. Nothing in the local vascular architecture of the distal median nerve is directly suggestive of any particular local predisposition to neural ischaemia, such as has been suggested as the cause of the carpal tunnel syndrome by Dorndorff (1931), Pritchard (1950) and Kremer, Gilliatt, Golding & Wilson (1953). It is relevant to note, however, that in traversing the carpal tunnel the median nerve lies in relatively avascular surroundings, and that the branches of the ulnar and radial arteries, and of the superficial palmar arch, supplying the median nerve ramify also on the anterior surface of the common synovial flexor sheath. Bentley & Schlapp

(1943) have demonstrated that the oxygen requirements of a nerve trunk may be appreciably met, after occlusion of its regional nutrient arteries, by oxygen diffusion from surrounding, well-vascularized tissues. It is possible, therefore, to envisage circumstances in which spasm of the nutrient arteries, including their branches to the common synovial flexor sheath, might result in a degree of neural anoxia at the distal end of the median nerve sufficient to impair nerve conduction.

SUMMARY

The gross blood supply and intrinsic vascular anatomy of the median nerve in the forearm and hand are described. The findings are discussed in relation to previous accounts of the arterial supply of the median nerve, and in relation to their clinical implications.

The author is grateful to Prof. A. J. E. Cave for advice and encouragement throughout the course of this investigation. Miss M. Shreeve has given valuable technical assistance. The photographs are the work of Mr A. E. Westwood, and the text-figure of Mrs A. Besterman.

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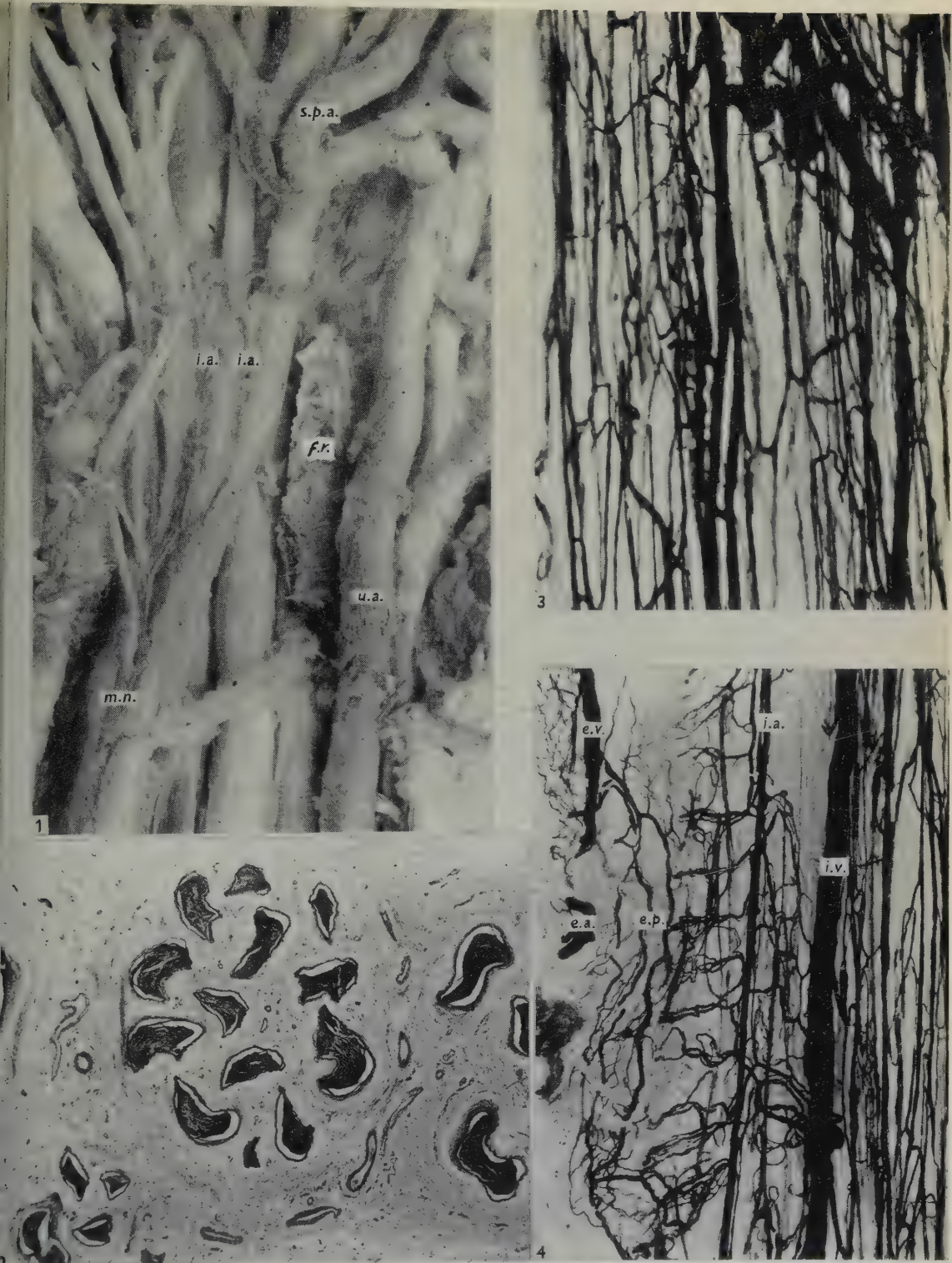
EXPLANATION OF PLATE

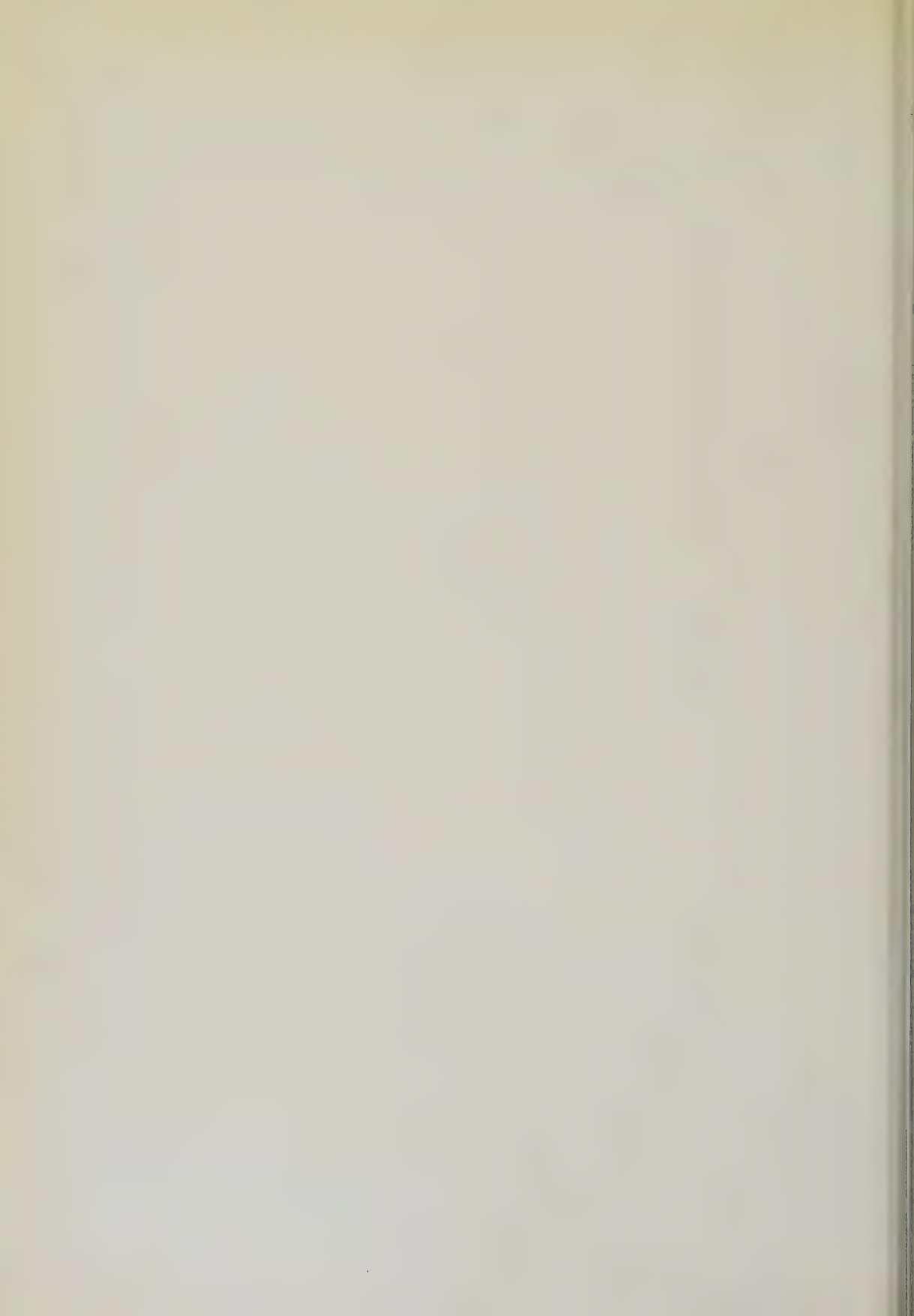
(Key to abbreviations used in Text-fig. 1 and in Pl. 1.)

<i>e.a.</i>	epineurial arteriole	<i>m.a.</i>	median artery
<i>e.p.</i>	surface epineurial capillary plexus	<i>m.n.</i>	median nerve
<i>e.v.</i>	epineurial venule	<i>r.a.</i>	radial artery
<i>f.r.</i>	flexor retinaculum	<i>s.p.a.</i>	superficial palmar arch
<i>i.a.</i>	interfascicular arteriole	<i>u.a.</i>	ulnar artery
<i>i.v.</i>	interfascicular venule		

PLATE 1

- Fig. 1. Fasciculi at the distal end of the median nerve have been separated to show interfascicular arteries derived from a branch of the superficial palmar arch. Neoprene injection.
- Fig. 2. Transverse section of median nerve at the lower border of the flexor retinaculum. Haematoxylin and Biebrich scarlet. $\times 22$.
- Fig. 3. Longitudinal section of median nerve showing intraneural vascular plexuses. Monastral fast blue injection. $\times 46$.
- Fig. 4. Longitudinal section of median nerve showing the surface epineurial capillary plexus. Monastral fast blue injection. $\times 50$.





DEVELOPMENTAL CHANGES IN THE INTERVENTRICULAR FORAMEN

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The changes taking place in the interventricular foramen, at the junction of the third and lateral cerebral ventricles, during ontogeny, have been studied previously by many workers. Interest has been directed mainly to the structures developed in the walls of the foramen, and little or no attention has been paid to the aperture of the foramen itself.

Streeter (1912) states that the interventricular foramen is produced 'not as an actual constriction but secondarily through the fact that its boundaries remain nearly stationary while the pallial walls undergo enormous expansion'.

This belief that the foramen becomes reduced in size only relatively was shown to be invalid in the case of the pig by Heuser (1913). He investigated the formation of the cerebral ventricles in pig embryos, and gives a series of measurements of the approximate area of the interventricular foramen at its narrowest point; his measurements clearly show a progressive reduction in the size of the foramen from 12 mm. c.r. length to 45 mm. c.r. length, with a subsequent re-expansion.

Hines (1922) also implies that the foramen undergoes some reduction, due to the growth of the structures immediately surrounding it, viz. the corpus striatum, thalamus, lamina terminalis and choroid plexus, but she does not support this statement by actual measurements of the area of the foramen.

Since Heuser published his observations on the pig, little attention has been paid to the possibility that the foramen undergoes actual constriction during early ontogeny; the aim of the present contribution is to examine the changes in the size of the interventricular foramen in embryos of a number of different species, in an attempt to confirm or disprove the findings of Heuser.

MATERIAL AND METHODS

A total of thirty-two embryos, belonging to five different species, formed the basis of this investigation. All had been serially sectioned after routine Bouin fixation and paraffin embedding. The embryos studied included the following:

Sheep	10, 11.5, 12, 13, 14, 16, 18.5, 20, 28 mm. c.r. length
Man	8, 14.5, 24, 29, 41 mm. c.r. length
Pig	10, 13, 17.5, 29, 36, 42.5 mm. c.r. length
Rat	5, 11, 15, 19, 22, 30 mm. c.r. length
Rabbit	8, 10, 16, 20, 30, 35 mm. c.r. length

The forebrains of five of the sheep embryos were reconstructed by the usual wax plate procedure, at a magnification of $\times 60$. All the human, pig, rat and rabbit embryos were used to produce graphic reconstructions of medial sagittal views of

the forebrain. Those sections which passed through the interventricular foramina enabled accurate measurements to be made of the area of the foramen. The correct proportion of sections from the total number making up the foramen (as calculated according to thickness and magnification, for the reconstructions) was projected at a known linear magnification. Direct measurements of the width of the foramen at its narrowest point in each section enabled the area of the foramen to be calculated; both foramina, right and left, were measured, and the mean of the two results obtained.

The possible effects of fixation and dehydration on the ultimate size of the embryo have been ignored. Thus all measurements are probably about 10 % below their value in life.

RESULTS

The areas of the interventricular foramina in the embryos examined are listed in Table 1. The measurements are presented in graphic form in Fig. 1.

In every case it can be seen that there is an undoubted diminution in the size of the interventricular foramen and, furthermore, it is evident that there is a period during which the constriction of the foramen is occurring with especial rapidity,

Table 1. *The area of the interventricular foramen in embryos of sheep, man, pig, rat and rabbit*

Species	c.r. length (mm.)	Area of foramen (mm. ²)
Sheep	11.5	0.57
	12.0	0.61
	13.0	0.63
	14.0	0.67
	16.0	0.71
	18.5	0.35
	20.0	0.25
	28.0	0.29
Man	8	0.72
	14.5	0.70
	24	0.09
	29	0.12
	41	0.15
Pig	10	0.44
	13	0.40
	17.5	0.16
	29	0.07
	36	0.14
	42.5	0.19
Rat	5	0.10
	11	0.027
	15	0.026
	19	0.006
	22	0.01
	30	0.01
Rabbit	8	0.42
	10	0.41
	16	0.088
	20	0.10
	30	0.028
	35	0.007

evidenced by the steepness of the graph. This rapid phase of constriction is confined to the following stages in the different species:

Sheep	16-18.5 mm. C.R. length
Man	15-25 mm. C.R. length
Pig	13-17.5 mm. C.R. length
Rat	5-10 mm. C.R. length
Rabbit	10-15 mm. C.R. length

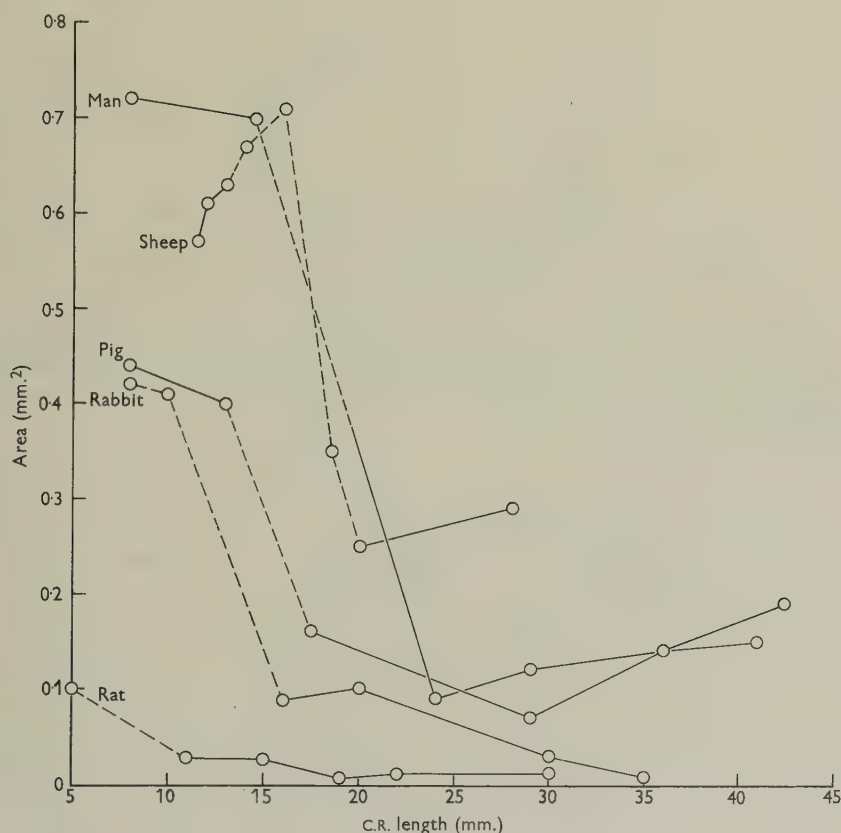


Fig. 1. The area of the interventricular foramen in sheep, man, pig, rat and rabbit. The portion of each graph represented in dotted lines indicates the period during which the caudal poles of the hemispheres are initially formed.

After this period of rapid diminution in size, the changes in the area of the foramen differ from species to species. In the sheep, the phase of constriction of the foramen is preceded, as well as succeeded, by periods of increase in its size. Similarly in man, there is tendency for the foramen to undergo re-expansion after constriction. In the pig this secondary increase in the area of the foramen is particularly well marked. In the two rodents, however, there is no evidence of such re-expansion in the embryos examined.

It is well known that the interventricular foramen, commencing as an approxi-

mately circular aperture at the time of the evagination of the hemisphere from the prosencephalon, becomes altered in shape as development proceeds. During the early stages, with which this investigation is concerned, one factor responsible for this change in shape is the growth of the corpus striatum in the floor of the foramen, which ultimately converts the initially circular opening into a curved slit. The variations in the outline of the foramen can be followed in Figs. 2 and 3, which

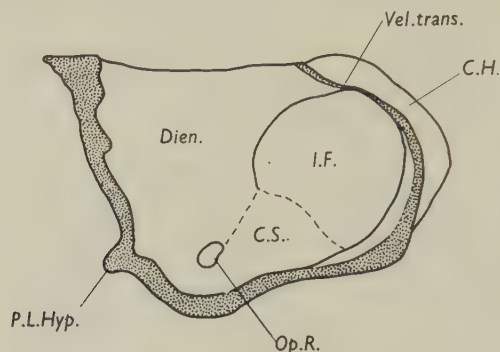


Fig. 2. 13 mm. sheep embryo. Outline diagram of left half of a wax plate model of the forebrain, medial aspect. *Vel.trans.*, velum transversum; *Dien.*, diencephalon; *I.F.*, interventricular foramen; *C.S.*, corpus striatum; *P.L.Hyp.*, posterior lobe of hypophysis; *Op.R.*, optic recess; *C.H.*, cerebral hemisphere.

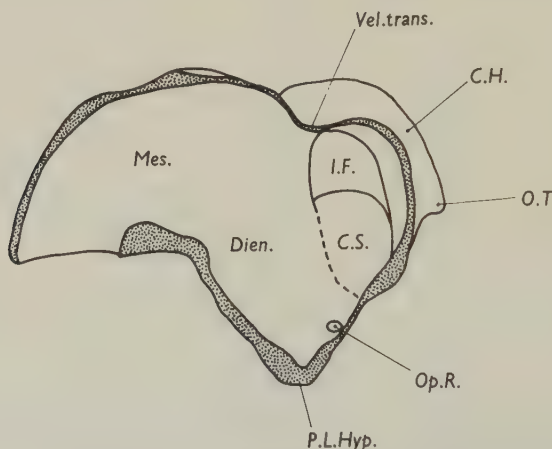


Fig. 3. 16 mm. sheep embryo. Outline diagram of left half of a wax plate model of the forebrain, medial aspect. *O.T.*, olfactory tubercle; *Mes.*, mesencephalon. Other abbreviations as in fig. 2.

present medial sagittal views of wax plate models of two of the sheep embryo forebrains. The foramen in a 13 mm. embryo is shown in Fig. 2; its outline is well established dorsally and rostrally, but is imperfectly seen ventrally, where the bulge of the corpus striatum is becoming apparent. Apart from this encroachment of the corpus striatum into the foramen, the aperture is essentially circular.

In Fig. 3, a 16 mm. embryo brain, the foramen exhibits two marked changes

when compared with the 13 mm. specimen. First, the corpus striatum has expanded rapidly and now occupies a considerable proportion of the foramen. Secondly, in that part of the foramen which remains widely open it can be seen that the aperture between the third and lateral ventricles has become narrower in the rostro-caudal plane, i.e. ignoring the presence of the corpus striatum, the foramen as a whole is no longer circular but essentially oval, with the long axis directed dorso-ventrally.

DISCUSSION

The measurements of the area of the interventricular foramen at its narrowest point in the pig embryos confirm the findings of Heuser (1913), and show marked constriction of the foramen. A similar constriction is apparent in the embryos of sheep, man, rat and rabbit, so that it can be stated that this constriction is not peculiar to the pig.

Since the occurrence of this constriction has generally been overlooked, correspondingly little attention has been paid to the mechanical factors which might be responsible for it. As already stated, Hines (1922) was aware of the possibility of a diminution in the size of the foramen, which she attributed to growth of the corpus striatum, thalamus, lamina terminalis and choroid plexus towards its centre. A study of the wax plate reconstructions of the sheep brains, taken in conjunction with the graph of the area of the foramen in the sheep (Fig. 1), indicates that, of these four structures, only the corpus striatum can play any active part in diminishing the size of the foramen during the period under consideration. The growth of the thalamus, choroid plexus and lamina terminalis, takes place some time after the phase of most rapid constriction of the foramen. Furthermore, the corpus striatum itself continues to grow rapidly after the rapid diminution of the foramen has ceased.

It would seem probable, therefore, that increase in size of the striatum is not the only factor responsible for the constriction of the interventricular foramen. Comparison of the wax models of the 13 and 16 mm. sheep brains (Figs. 2, 3) suggest very strongly that the foramen is not only being invaded from below by the corpus striatum, but that it is also undergoing a fundamental change in its shape, presenting an initially circular outline which by the 16 mm. stage has been converted to an oval, whose long axis lies dorso-ventrally

An explanation of this change in the shape of the foramen may be found in a suggestion recently made by Droogleever Fortuyn (1955, 1956). Fortuyn maintains that the commonly accepted explanation of the mode of formation of the freely projecting caudal poles of the hemispheres by caudal evagination of the telencephalon is incorrect. He believes that invagination of the rostral end of the diencephalon into the caudal end of the telencephalon is responsible for the production of the caudal poles of the hemispheres. This is illustrated diagrammatically in Fig. 4.

If it be assumed that Fortuyn is correct, it follows that during the invagination of the diencephalon into the telencephalon there should be resultant changes in the interventricular foramen, affecting both its shape and its size, since the mechanism which he proposes would involve a rostral movement of the caudal (thalamic) boundary of the foramen, tending first to reduce the size of the foramen and secondly to render it more oval in shape. It has already been shown here that such

an alteration in the shape of the foramen is indeed detectable, so that the foramen as a whole shows evidence of narrowing in the rostro-caudal plane. It can further be demonstrated that the phase of most rapid constriction of the foramen, in all the five species studied, occurs largely within the period during which the initial formation of the caudal poles of the hemispheres is taking place.

The stages of development during which the freely projecting caudal end of the hemisphere appears, have been established from the reconstructions of the fore-brains of the species examined. They are as follows:

Sheep	13-20 mm. C.R. length
Man	14.5-19.5 mm. C.R. length
Pig	13-17.5 mm. C.R. length
Rat	5-11 mm. C.R. length
Rabbit	8-16 mm. C.R. length

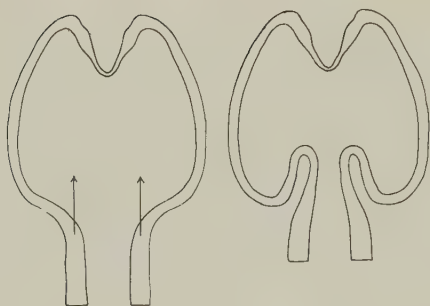


Fig. 4

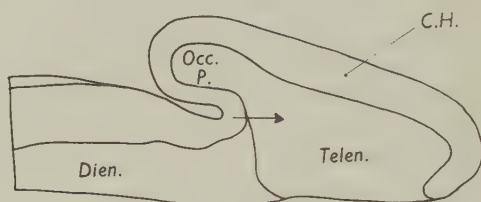


Fig. 5

Fig. 4. Diagram to illustrate Fortuyn's idea of the formation of the freely projecting caudal poles of the hemispheres. On the left, the caudal poles have not been established. The arrows indicate that the rostral end of the diencephalon is about to be pushed rostrally into the telencephalon, thus constricting the interventricular foramen. On the right the process has been completed, the caudal poles produced and the foramen constricted.

Fig. 5. 16 mm. sheep embryo. Outline diagram of wax plate model of dorsal portion of di-telencephalic junction. The arrow marks the point at which the rostral end of the diencephalon (Dien.) is being thrust into the caudal end of the telencephalon (Telen.). C.H., cerebral hemisphere; Occ.P., occipital pole.

Comparison of these figures with those for the period of most rapid constriction of the interventricular foramen reveals quite close agreement between the two. This can also be seen in the graphs (Fig. 1) in which the dotted portion of each graph indicates the time during which the caudal poles of the hemispheres are first appearing. In each case this corresponds quite closely to the steepest part of the graph.

Finally, in Fig. 5 it can be seen that the configuration of the brain wall at the di-telencephalic junction is quite in keeping with the possibility of the invagination of the diencephalon into the telencephalon.

Thus the changes in the size and shape of the interventricular foramen which have been described here seem to be in accordance with those which might be predicted on the basis of Fortuyn's hypothesis.

SUMMARY

1. Measurements have been made of the area of the interventricular foramen at its narrowest point in young embryos of sheep, man, pig, rat and rabbit. In each of these animals there is distinct and rapid constriction of the foramen during the early phases of development, as originally reported in the pig by Heuser (1913).

2. Examination of wax plate models of the forebrain of sheep embryos reveals that the constriction of the foramen is accompanied by a change in its shape, from circular to oval.

3. The period during which the foramen is undergoing its most rapid constriction synchronizes with the initial formation of the freely projecting caudal poles of the hemispheres.

4. The mechanical factors responsible for these changes in the interventricular foramen include (1) the growth of the corpus striatum, (2) invagination of the diencephalon into the telencephalon, as suggested by Fortuyn.

I should like to acknowledge my indebtedness to Prof. A. Durward, for his guidance and encouragement throughout this investigation.

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CARDIAC MUSCLE RELATIONS OF THE CORONARY SINUS, THE OBLIQUE VEIN OF THE LEFT ATRIUM AND THE LEFT PRECAVAL VEIN IN MAMMALS

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INTRODUCTION

Many authors (reviewed by Lechner, 1942) have noted the presence of striated cardiac muscle in the walls of the pulmonary veins and the right precaval vein (superior vena cava) near their entrance to the heart. Few workers have made similar studies of the coronary sinus (Keith, 1902; Tandler, 1913; Papez, 1920; Lechner, 1942), and there is considerable divergence of opinion among these investigators. No such study of the left precaval vein or the oblique vein of the left atrium appears to have been carried out. During a recent study of the atrial cardiac ganglia in many mammals, including man, King & Coakley (1958) noted the presence of cardiac muscle in the oblique vein of the left atrium and the left precaval vein, and in some specimens it was observed that many of the fibres of this musculature closely resemble histologically the fibres of the sinu-atrial (s.a.) and atrioventricular (a.v.) nodes. In the present work a more detailed investigation of the relation of the cardiac muscle to the coronary sinus, the left precaval and the oblique vein has been made.

MATERIALS AND METHODS

The twenty-seven hearts examined comprised the following, the number of hearts from each species being indicated:

Specimens with left precaval vein: platypus (*Ornithorhynchus anatinus*, 1), wallaroo (*Macropus robustus rubens*, 1), hedgehog (*Erinaceus europaeus*, 1), mole (*Talpa europaea*, 1), rabbit (*Oryctolagus cuniculus*, 1), laboratory rat (*Rattus norvegicus*, 2), fruit bat (*Pteropus* sp., 1), tree shrew (*Tupaia* sp., 1).

Specimens with oblique vein of left atrium: guinea-pig (*Cavia porcellus*, 2), cat (*Felis domesticus*, 2), dog (*Canis canis*, 2), porpoise (*Phocaena phocaena*, 1), pig (*Sus scrofa*, 2), domestic ox (*Bos taurus*, 3), sheep (*Ovis aries*, 1), horse (*Equus caballus*, 1), rhesus monkey (*Macacus mulatta*, 1), man (*Homo sapiens*, 3; 1 adult and 2 infants).

The animals listed above are divided into two groups according to the type of vein present in the particular hearts examined. It obviously could be maintained that, because so few specimens of each species were examined, it is not possible or safe to be dogmatic as to whether the type of vein observed is normally present in a particular species or whether it is a variation or abnormal arrangement in the individual heart examined. A survey of the literature, however, indicates that in those of our specimens that have a left precaval vein, this feature appears to be

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normal for these animals, e.g. platypus, wallaroo, hedgehog, mole and many rodents (Weber, 1904*a*; Benninghoff, 1933), hedgehog and mole (Dobson, 1882), rabbit (Krauze, 1868), rat (Greene, 1955), fruit bat (Grosser, 1901) and tree shrew (Weber, 1904*b*). Again, an oblique vein of the left atrium is stated to be the usual arrangement in the guinea-pig (James, 1904), cat (Reighard & Jennings, 1935), dog (Bradley, 1943), Cetacea and Ungulates (Weber, 1904*a*), horse (Chauveau, 1891) and Primates (Weber, 1904*a*).

In most cases, the entire heart was embedded in paraffin and serially sectioned in a plane parallel to the A.V. sulcus. From the hearts of the horse and pig, blocks of tissue were removed and paraffin or frozen sections prepared. The staining techniques were haematoxylin and eosin, iron haematoxylin and picrofuchsin (van Gieson), Masson's trichrome stain (using light green), pyridine-silver, and the Ranson, Smith-Quigley and Bielschowsky-Gros silver methods.

OBSERVATIONS

The standardized diagrams in Text-fig. 1 show the atria viewed from the cranial aspect and depict the left precaval vein (A) and the two types of oblique vein (B, C) observed in the present study. The dots indicate the position of the subepicardial ganglia previously investigated (King & Coakley, 1958). Numerous ganglia and nerves were found to accompany the left precaval vein, the oblique vein and the coronary sinus.

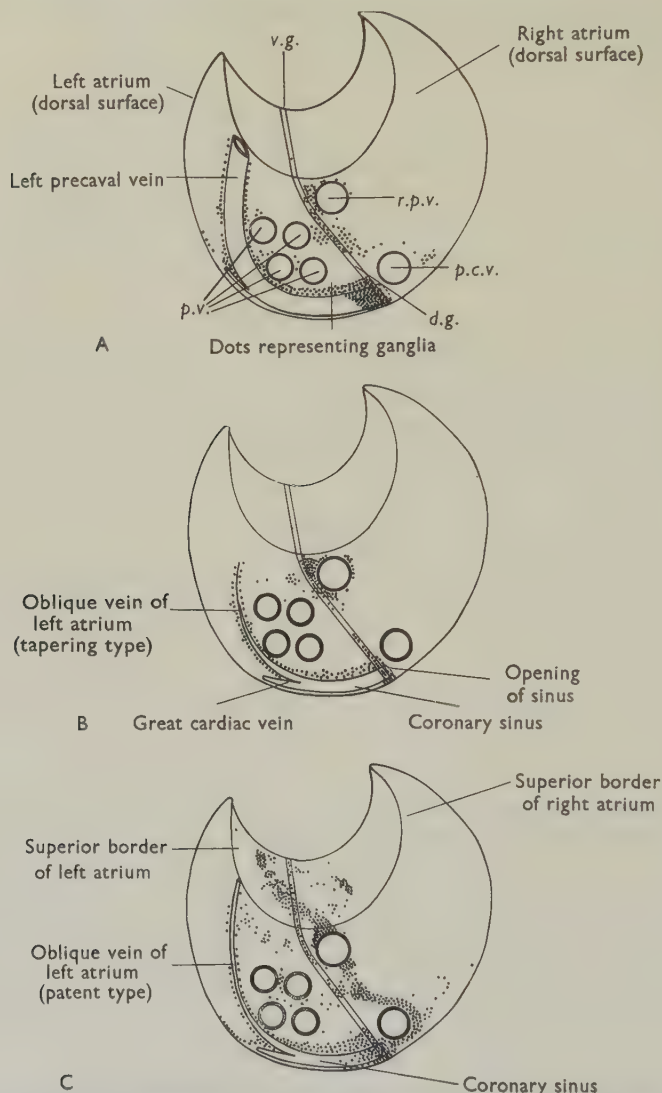
The patent type of oblique vein (Text-fig. 1 C) present in our specimens in the pig, ox, sheep and horse, extends cranially as a narrow patent vessel beyond the atrium, while the tapering type (Text-fig. 1 B), in the guinea-pig, cat, dog, porpoise, rhesus monkey and man, becomes very slender and ends blindly on the dorsal wall of the left atrium. In all the hearts, the left extremity of the coronary sinus is considered to be the site of junction of the left (great) cardiac vein with the oblique vein or the left precaval vein.

In all the hearts, cardiac muscle was found to be closely associated with the coronary sinus and the left precaval or oblique vein. In some hearts this muscle is histologically identical with ordinary cardiac muscle, but in the hedgehog, mole, rabbit, guinea-pig, cat, dog, pig, horse, tree shrew and rhesus monkey many of the muscle fibres are narrower, paler staining and less distinctly striated than those of the neighbouring atrial myocardium, and in these respects they resemble the fibres of the S.A. and A.V. nodes (Pl. 1, fig. 6). These fibres are found throughout the whole length of the coronary sinus but are limited to the caudal half or less of the oblique vein and left precaval vein. In the guinea-pig, there is in addition a mass of these 'nodal' fibres on the dorsal wall of the left atrium, mainly lying dorsal to the caudal half of the oblique vein. It is about one-third of the transverse width of the atrium and the fibres become continuous cranially and caudally with those in the wall of the vein.

The arrangement of the cardiac muscle associated with these veins varies according to the type of vein present.

The left precaval vein has a complete coat of cardiac muscle, the fibres being arranged in a circular or spiral fashion and replacing the venous tunica adventitia and media (Pl. 1, fig. 1). Although in general these fibres are separate from the

atrial myocardium, at a few places continuity is established. The cardiac muscle coat extends cranially beyond the limits of the specimens (i.e. beyond the cranial border of the left atrium). Caudally it is continuous with the cardiac muscle related to the coronary sinus, which is arranged in a similar manner (Pl. 2, fig. 5), and which in



Text-fig. 1. Standardized diagrams of cardiac atria of tree shrew (A), guinea-pig (B) and domestic ox (C). *d.g.*, dorsal interatrial groove; *p.c.v.*, postcaval vein; *p.v.*, pulmonary veins; *r.p.v.*, right precaval vein; *v.g.*, ventral interatrial groove.

turn, at the entry of the sinus into the right atrium, becomes continuous with the myocardium of the atrium and with the fibres of the A.V. node, this latter continuity involving both the 'nodal' fibres and the ordinary cardiac muscle in the wall of the sinus.

In those hearts with the patent type of oblique vein the cardiac muscle related to both the vein and the coronary sinus has the same arrangements as in those hearts with a left precaval vein (Pl. 1, fig. 2).

In the case of the tapering type of oblique vein the cardiac muscle arrangements are different and vary somewhat in the different animals. In general the cardiac muscle lies outside the venous tunica adventitia and media and does not form a compact and complete coat around the vein (Pl. 1, fig. 4). For the most part it is quite separate from the myocardium of the left atrium, though continuity is established at a number of points along the course of the vein. Most of the fibres run parallel to the vein but a few of them form rings or spirals around the nerves accompanying the vein. In the guinea-pig, cat and porpoise the muscle forms a loose network around or beside the vein (Pl. 1, fig. 4), and while in one dog this arrangement was present, in the other the muscle formed a compact bundle close beside the vein. In the rhesus monkey (Pl. 1, fig. 3) the muscle forms rounded bundles encircling the vein, while in man it consists of strands intermingled with nerves, ganglia and subepicardial connective tissue. Cranially, the cardiac muscle fibres taper and end blindly in the subepicardial connective tissue beyond the vein, though in man most of them become continuous with the myocardium of the dorsal wall of the left atrium. Caudally, the muscle fibres become continuous with the cardiac muscle related to the coronary sinus, which forms a complete coat around the sinus and ends in the same manner as described above in the hearts with a left precaval or patent type of oblique vein.

Thus in all the hearts, the cardiac muscle arrangement in relation to the coronary sinus is the same. While the arrangement of the muscle in relation to the left precaval vein and the patent and tapering types of oblique vein varies with the type of vein, the presence or otherwise of 'nodal' fibres in these veins does not appear to be so related. In all these veins, numerous nerve fibres, both medullated and non-medullated lie among the muscle fibres.

DISCUSSION

In all the hearts studied the cardiac muscle related to the coronary sinus has a constant arrangement; namely that it extends along the entire length of the sinus and forms a complete (for the main part circular) coat replacing the tunica adventitia and media found in veins generally. The arrangement in the other veins studied varies according to the type of vein present. In the left precaval vein and the patent type of oblique vein, the cardiac muscle extends beyond the cranial border of the left atrium (i.e. outside the limits of the heart specimens) and is arranged in the wall of these veins in the same manner as in the coronary sinus. In the tapering type of oblique vein, the cardiac muscle lies outside the tunica adventitia of the vein, is sparser and does not form a complete coat for the vein. So far as the coronary sinus is concerned, these results are at variance with those of previous workers. Lechner (1942) found considerable variation among species; e.g. in the coronary sinus he found no cardiac muscle in the ox, none or only at the termination of the sinus in rodents, only at the end of the sinus in marsupials, a partial covering in the cat, and extending only for a short distance along the sinus in insectivores. Extension of cardiac muscle into the wall of the sinus in man has been noted by Keith (1902),

Tandler (1913) and Papez (1920). Papez observed that in the bovine heart the coronary sinus is surrounded by cardiac muscle for a distance of several inches.

In a number of the animals studied in the present work, many of the muscle fibres related to the coronary sinus and the left precaval or oblique vein were found to resemble the fibres of the s.a. and a.v. nodes. With regard to the significance of these fibres, the work of Shaner (1929) and Jones (1932) is pertinent. These authors described the temporary appearance of a left a.v. node in the embryo of the calf (Shaner) and human (Jones), and it might be considered that the muscle fibres in the present series may represent the persistence of such a structure. On the other hand, from their position in relation to the coronary sinus which is developed from the left horn of the sinus venosus, they may be regarded as analogous to the normal s.a. node related to the junction of the right precaval vein with the part of the right atrium derived from the right horn of the sinus venosus, and in this respect they might be considered as a left s.a. node. However, the fact that in all cases the fibres are directly continuous with those of the 'normal' a.v. node renders it more probable that they should be regarded merely as an extension of the latter into the walls of the veins.

SUMMARY

1. Several mammalian hearts have been examined histologically to ascertain the cardiac muscle relationships of the coronary sinus and of the left precaval vein or the oblique vein of the left atrium.

2. Two types of oblique vein are described, the patent and the tapering type.

3. In all specimens the coronary sinus is completely covered by striated cardiac muscle which replaces the tunica adventitia and media.

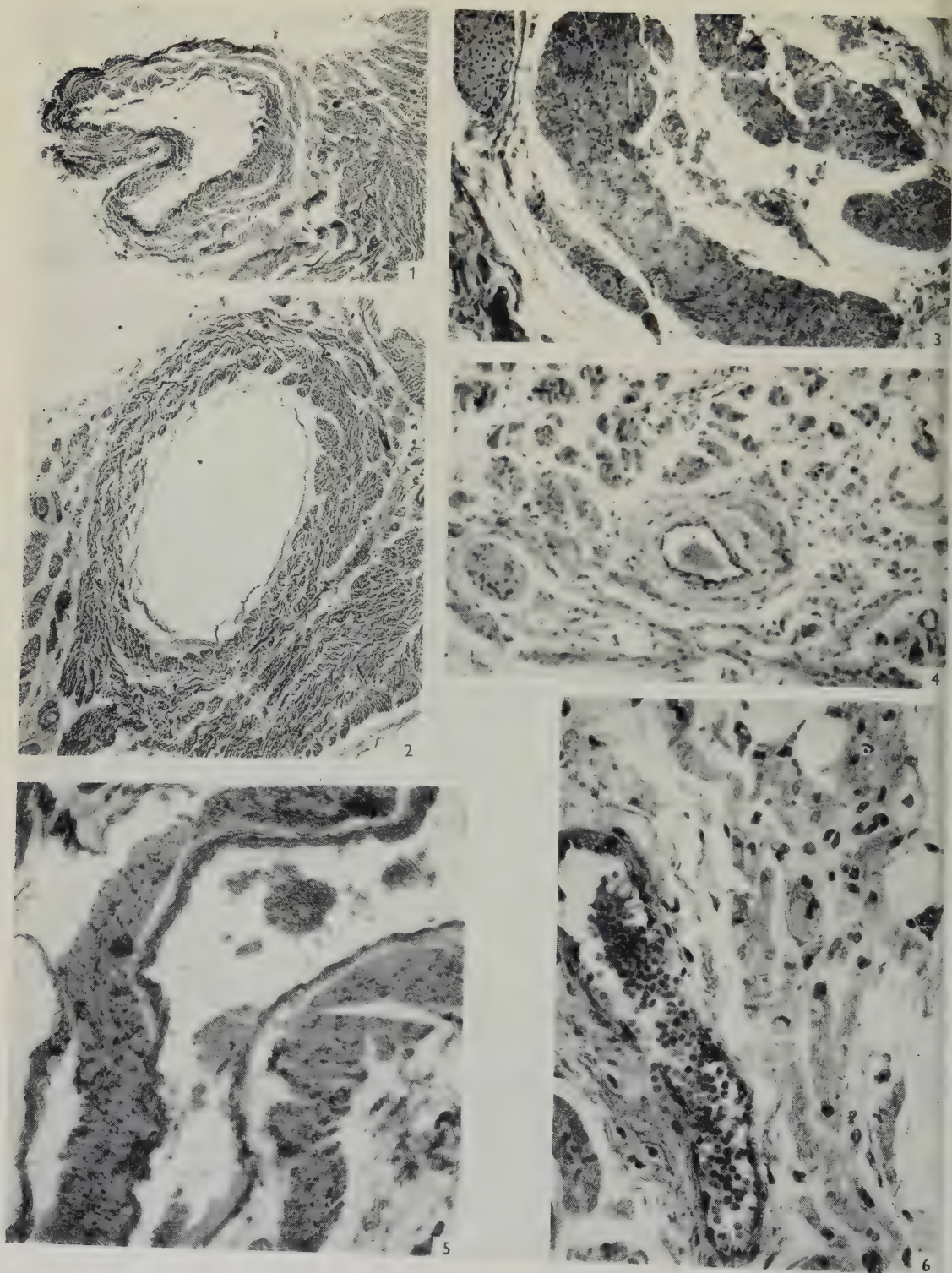
4. The left precaval vein and the patent type of oblique vein have the same cardiac muscle arrangements as the coronary sinus, whereas the tapering type of oblique vein has a sparser and less complete cardiac muscle coat which lies outside the adventitia of the vein.

5. In some of the hearts many of the muscle fibres in the wall of the coronary sinus and of the left precaval or oblique vein resemble the fibres of the s.a. and a.v. nodes and are continuous with the a.v. nodal fibres. They are considered to be extensions of the a.v. node into the walls of the veins.

We wish to acknowledge a grant to one of us (T.S.K.) from the University of Sheffield Medical Research Fund.

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EXPLANATION OF PLATE

(All the sections illustrated were stained with haematoxylin and eosin.)

- Fig. 1. Transverse section of left precaval vein in the rabbit. $\times 25$. Note the cardiac muscle replacing the adventitia and media of the vein.
- Fig. 2. Transverse section of oblique vein (patent type) in the sheep. $\times 18$. Note the cardiac muscle replacing the adventitia and media of the vein.
- Fig. 3. Transverse section of oblique vein (tapering type) in the rhesus monkey. $\times 100$. Note the cardiac muscle bundles outside the adventitia of the vein.
- Fig. 4. Transverse section of oblique vein (tapering type) in the guinea-pig. $\times 130$. Note loose arrangements of cardiac muscle around and outside the adventitia of the vein.
- Fig. 5. Longitudinal section of coronary sinus in the rhesus monkey. $\times 70$. Note the cardiac muscle replacing the adventitia and media of the vein.
- Fig. 6. Longitudinal section through the caudal end of the oblique vein in the guinea-pig. $\times 300$. Note the pale-staining 'nodal' fibres on the right compared with the ordinary atrial myocardium (bottom left).

HISTOCHEMICAL OBSERVATIONS ON THE FOETAL VAGINAL EPITHELIUM

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A diastase-resistant periodic acid-Schiff reaction was described by Wislocki, Fawcett & Dempsey (1951) in the intercellular material of the more superficial layers of the stratified squamous epithelium of certain mucous membranes, including the mucous membrane of the vagina. This they attributed either to glycogen which was so firmly bound to protein that it could not be removed by salivary digestion, or to a mucopolysaccharide. In the vaginal epithelium of two human foetuses, of 180 and 375 mm. C.R. length respectively, a similar diastase-resistant PAS reaction has been found, confined to the 'intercellular' material of the intermediate and superficial layers. This material is a prominent feature of ordinary histological preparations of the vaginal epithelium, and gives the appearance of a honeycomb arrangement of walls dividing adjacent cells from each other. It is possible that these 'walls' do consist of intercellular material. On the other hand, it may be that they are condensations of the peripheral cytoplasm of the cells themselves, and because of this uncertainty it is difficult to find a satisfactory terminology. The name 'intercellular walls' will be used here for convenience, but it is not intended to imply any precise relation of these structures to the epithelial cells.

OBSERVATIONS

The present work is a study of certain histochemical features of the intercellular walls of the vaginal epithelium in these two human foetuses. Though only formalin-fixed paraffin sections have been available, the results obtained nevertheless seem sufficiently significant to be recorded.

It will first be necessary to give a brief account of the histological structure, as seen in sections stained by a trichrome technique (haematoxylin, ponceau de xyridene and aniline blue). In the 180 mm. foetus (Pl. 1, fig. 1) the epithelium is very thick, comprising up to thirty layers of cells, and it can be conveniently described in three zones. The basal zone is formed by two or three layers of small cells, with basophil cytoplasm and relatively large nuclei. The intermediate zone consists of about six or seven layers of larger and flattened cells, with smaller nuclei. Here the intercellular walls become very obvious and are stained a brownish colour in the trichrome preparation. The cytoplasm is usually clear, but is sometimes stained a pale brownish colour in the deeper layers of cells. In the basal layers of this zone intercellular bridges can be identified, crossing the intercellular walls, but they are much less prominent than in the epidermis. The superficial zone is very thick, with up to twenty layers of large cells, often less flattened than the cells of the intermediate zone. The cytoplasm is completely clear except for a few scattered basophil granules.

The intercellular walls are very pronounced, and the nuclei either very small and lacking in chromatin, or completely absent.

In the 375 mm. foetus the epithelium is considerably thinner (Pl. 1, fig. 2). The basal zone is formed by three or four layers of small cells, similar to those of the basal zone of the 180 mm. foetus. This is surmounted by four or five layers of large cells, with clear-staining cytoplasm and fairly large nuclei. The intercellular walls are first apparent in this zone, and intercellular bridges can be seen crossing them. The superficial zone consists usually of three or four layers, in which the cells tend to be more flattened, particularly as they approach the surface. The intercellular walls are more distinct, intercellular bridges can no longer be identified, and the nuclei are smaller. The vaginal lumen is filled with a large amount of epithelial debris, implying a massive desquamation since the stage represented by the younger foetus.

As has been pointed out, the intercellular walls give a strongly positive PAS reaction, persisting after digestion with saliva or malt diastase sufficient to abolish the intense reaction of the intracellular glycogen of the intermediate and superficial zones (Pl. 1, fig. 3). In the basal zone, where intercellular walls cannot be seen in the trichrome preparation, the PAS reaction is negative.

Sudan black B in 70 % alcohol gives well-marked staining of the intercellular walls of the intermediate and superficial zones (Pl. 1, fig. 4). In addition, the nuclei are faintly stained, and the cytoplasm of the basal zone of cells shows an overall staining absent from the more superficial cells. Rapid removal of the stain in alcohol or acetone, and the ease with which it can subsequently be replaced, indicate that the sudanophilia is due to lipid material which has persisted through the processes of fixation and embedding.

As might be expected, because of the lipid content, positive reactions are obtained with the peracetic and performic acid-Schiff techniques. For both these reactions the methods of Pearse (1953) and Lillie (1954) were employed, with their differing preparations of the peracid reagents, and it was found that the peracetic acid of Lillie's method was much more effective in producing a reaction than that prepared by Pearse's method. The performic acid of Lillie's method also seemed more satisfactory, since a positive reaction could be obtained with Schiff's reagent after treatment with formic acid alone. While this reaction was very weak in the dilute solution of formic acid corresponding to Lillie's performic acid reagent, it was much stronger in a solution with the higher formic acid content of Pearse's reagent. It seems, therefore, that a part of the performic acid-Schiff reaction of lipid substances, when carried out according to Pearse's directions, is due to formic acid and not performic acid. With both techniques, however, the PAAS and PFAS reactions of the foetal vaginal epithelium are positive in the intercellular walls, in the nuclei and in the cytoplasm of the basal cells, in a similar distribution to the sudanophilia (Pl. 1, fig. 5). The reaction of the nuclei is more marked, however, and is in part, presumably, a Feulgen reaction.

Either no reaction, or a negligible reaction, is obtained with 30 min. direct exposure to Schiff's reagent, and this is not appreciably enhanced by 15 min. prior treatment with either 2 % mercuric chloride solution or 0.1 N hydrochloric acid. A considerable reaction, less marked than after peracetic or performic acid oxidation but with the same distribution except for less pronounced nuclear staining, resulted

from the direct immersion in Schiff's reagent of a section which had been hydrated and exposed to atmospheric oxidation for a few days under a thin layer of water. This autoxidation-Schiff reaction can be very much hastened and augmented by concurrent exposure to ultra-violet radiation.

Table 1. *The effects of blockade techniques on the histochemical reactions of the intercellular walls of the vaginal epithelium*

PAS reaction	++	Acetylation* Abolished (Restored by de-acetylation)§	Bromination† Unaffected	Dimedone‡ Abolished
PFAS and PAAS reactions	+	Unaffected	Reduced	Abolished
Autoxidation-Schiff reaction	+	Unaffected	Reduced	Abolished

* 6 hr. in 40 % acetic anhydride in pyridine.

† 1 hr. in 2.5 % bromine in carbon tetrachloride.

‡ 3 hr. in a saturated solution of dimedone in 5 % acetic acid at 60° C., controlled by exposure of another section to 5 % acetic acid alone at the same temperature.

§ Overnight treatment in alcoholic ammonia (Lillie, 1954).

Various blockade techniques were applied to the reactions with Schiff's reagent, and the main results of these are shown in Table 1. The PAS reaction is abolished by acetylation and restored by de-acetylation, and is apparently due, therefore, to the presence of 1, 2-glycols or their amino- or keto-derivatives. The PAAS, PFAS and autoxidation-Schiff reactions are not abolished by acetylation, bromination, or by acetylation and bromination. A considerable reduction of the reaction occurs, however, after bromination, and this is accompanied by a marked Feulgen reaction in the nuclei. The PAS, PAAS, PFAS and autoxidation-Schiff reactions are all completely blocked by treatment for 3 hr. at 60° C. with either 5 % phenylhydrazine hydrochloride or 25 % hydroxylamine hydrochloride. A dimedone blockade completely abolishes the PAS, PFAS, PAAS and autoxidation-Schiff reactions of the intercellular walls, but does not affect the PAS reaction of the intracellular glycogen, and this can provide a convenient method for the positive visualization of glycogen in a PAS preparation.

Repeated attempts were made to remove the sudanophilic material from the intercellular walls with various fat solvents, but none of these was completely successful. A 24 hr. exposure to boiling methanol-chloroform, followed by extraction in alcohol-ether, effected a very considerable reduction of the sudanophilia and the PFAS reaction, however, without any apparent reduction in the intensity of the PAS reaction. It is likely, therefore, that the PAS reaction of the intercellular walls is due to some component other than the lipoid substance. The differing effects of the dimedone blockade on the intercellular walls and the intracellular glycogen indicate that this component is not bound glycogen. The most likely possibility would seem, therefore, to be a mucopolysaccharide, and various attempts were made to demonstrate such a material in the intercellular walls by other techniques. Sulphation in pure sulphuric acid, sulphuric acid in glacial acetic acid (Moore & Schoenberg, 1957) and sulphuric acid in ether (Gomori, 1954) all fail to induce a metachromatic basophilia in the intercellular walls after staining with toluidine

blue. On the other hand, the intracellular glycogen in the basal layers of the intermediate zone of the epithelium—the situation where glycogen is most abundant—is readily sulphated. Similarly, oxidation with 10% chromic acid for the various times used by Burkl (1953), followed by staining with toluidine blue, shows no reaction in the intercellular walls, while the intracellular glycogen in the basal layers of the intermediate zone stains metachromatically. Staining with 0.5% alcian blue 8G in 0.5% acetic acid, after the method of Lison (1954), produces marked staining of the intercellular walls, particularly in the most superficial layers, and also a faint staining of the intracellular glycogen. The only positive evidence for the presence of a mucopolysaccharide in the intercellular walls is given therefore by the PAS reaction, undiminished by salivary digestion and fat extraction, and the staining with alcian blue. This latter cannot, however, be regarded as of histochemical value.

The possibility was considered that the PAAS and PFAS reactions of the intercellular walls might be due to the presence of disulphide groups, either present in the original tissue or resulting from the oxidation of sulphydryl groups during the processes of embedding. After a prior treatment with Gram's iodine (Gomori, 1956), the intercellular walls show a moderate reduction of ferric ferriocyanide, but when the sections are exposed to a 10% solution of sodium thioglycollate before immersion in the ferriocyanide reagent the reaction is very much increased (Pl. 1, fig. 6). Both these reactions are abolished almost completely by a blockade of the reacting sulphydryl groups with a saturated solution of phenyl mercuric chloride in butanol (Pearse, 1953). Apparently, therefore, the intercellular walls in the sections which were used contain protein-bound sulphydryl and disulphide groups, though many of the latter may have been formed by the oxidation of sulphydryl groups during the preparation of the sections. The possibility existed that these disulphide groups might be partly responsible for the PAAS and PFAS reactions of the intercellular walls, in the manner in which Pearse (1953) explained the intense PAAS and PFAS reactions of hair shafts. In investigating this problem it was convenient that the sections of the vaginal epithelium also included the perineal skin, so that the reactions of the intercellular walls of the vaginal epithelium could be compared with those of the hair shafts in the same sections. The hair shafts give very strongly positive reactions with the PAAS and PFAS techniques, but, unlike the intercellular walls, also stain with methylene blue at pH 2.6 after oxidation with peracetic or performic acid. Their PAAS and PFAS reactions are almost completely abolished by bromination, but are unaffected by a 3 hr. dimedone blockade. The hair shafts are not sudanophilic, and give no autooxidation-Schiff reaction. Even if the PAAS and PFAS reactions of the hair shafts are due to the oxidation of disulphide groups, though Lillie & Bangle (1954) do not accept this view, these reactions are different in their behaviour from those of the intercellular walls. There is no evidence that the PAAS and PFAS reactions of the intercellular walls are in any degree due to the oxidation of disulphide groups to form a Schiff-positive substance.

Since the material was formalin-fixed, it was considered possible that some part of the reactions with Schiff's reagent might be due to the unmasking of the carbonyl groups of a lipid-formaldehyde compound such as Wolman & Greco (1952) suggested, though chemically it would seem improbable that formaldehyde could enter into a combination which leaves its carbonyl group free. Paraffin sections were

brought down to water, immersed in formalin, washed in running water and exposed to Schiff's reagent. An intense reddish purple colour was produced in the intercellular walls of the vaginal epithelium, while other tissues remained unstained. The colour was completely removed within an hour by washing in running water, but if the section was then re-immersed in Schiff's reagent the colour was partly restored. If, however, the washing was prolonged to several hours before re-immersion in the Schiff's reagent, or before the original immersion in Schiff's reagent, the only reaction was a very weak replica of the autoxidation-Schiff reaction. In paraffin sections, therefore, the lipoid-formaldehyde complex may be broken down by prolonged washing in water, and it seems unlikely to be a chemical compound. The reaction with Schiff's reagent, when the colour is washed out by running water, is similar to that of formaldehyde itself, and is quite unlike any of the reactions with Schiff's reagent after the various oxidation techniques which have been described here. There is no evidence that any of these reactions are the result of the original formalin fixation.

In an attempt to characterize further the lipoid material of the intercellular walls certain other techniques were employed. With Nile blue sulphate a blue staining was obtained, and this was completely removed by acetone; with Mallory's haemofuscin method the fuchsin was retained in 95 % alcohol; Gomori's chrome-haematoxylin method and Pearse's technique for the demonstration of phospholipids (1955) both produced negative results, and, in addition, the intercellular walls were not acid-fast. The lipoid material seems, therefore, to have some affinities with the group of lipofuscins, and this view receives support from the observation that in unstained preparations the intercellular walls are of a pale brownish colour. So little is known of the chemistry of the lipofuscins, however, that this association is probably not very helpful.

CONCLUSIONS

It has been established that there are two types of material in the intercellular walls of the vaginal epithelium in these two foetuses—a lipoid substance, and a protein which contains sulphhydryl and disulphide groups. In addition, it is probable that there is a mucopolysaccharide element which is responsible for the diastase-fast PAS reaction, though the positive evidence for this is incomplete. From the finding by Wislocki *et al.* (1951) of a positive PAS reaction in the intercellular material of the stratified squamous epithelium of other mucous membranes, and from Kollmann and Papin's depiction (1914) of osmiophil cell membranes in the oesophageal epithelium, it seems likely that these intercellular walls are typical of stratified squamous mucous membranes. No such structures are described, however, in the stratified squamous epithelium of the epidermis, and this seems to constitute an important difference between the keratinizing and non-keratinizing types of stratified squamous epithelium.

The lipoid material is presumably bound to the protein, which would account for its persistence in paraffin sections, and the autoxidation-Schiff reaction indicates its unsaturation. The failure of bromination to abolish completely the PAAS, PFAS and autoxidation-Schiff reactions might suggest that the lipoid is already in a partially peroxidized state, though there may be other explanations for the failure

of complete bromination of an unsaturated lipid. Accounts by organic chemists of the oxidation of unsaturated fats (Markley, 1947; Swern, 1953) do not describe aldehydes among the principal reaction products. However, the efficacy of the blockade with dimedone, a substance which is, according to Wild (1947), specific for aldehydes, implies their responsibility for the reactions which occur with Schiff's reagent after the oxidation of the intercellular walls of the foetal vaginal epithelium by organic peracids or by atmospheric oxidation. On the other hand, Cain (1949) has suggested that dimedone may produce some of its blocking effects by a reducing action.

The ferric ferri-cyanide reaction can only be considered to demonstrate the presence of the total of disulphide and sulphydryl groups. The presence of these groups in the 'cell walls' of the adult vaginal epithelium has been described by Asscher, Turner & de Boer (1956), and, in comparison with their work, it is of some interest that in the material of the present investigation the vaginal smear stain MF4 (Edward Gurr Ltd.) produced a uniform green staining of the intercellular walls of the vaginal epithelium. In the superficial layers of the intermediate zone of the 180 mm. foetus, however, the cytoplasm stained a bright orange colour, and in this position sulphydryl or disulphide groups were not demonstrated by the ferri-cyanide technique. The orange staining was prevented by a previous deamination, but not by acetylation.

The presence of a neutral mucopolysaccharide seems to be the most likely explanation of the PAS reaction of the intercellular walls, since there is no staining with 0.0005 M methylene blue at a pH lower than 4.6. The positive evidence for the presence of a mucopolysaccharide is, however, very slender, and it is possible that the PAS reaction is due to a glycolipid which persisted through all the methods of fat extraction which were employed and was responsible for the residual sudanophilia. It might even be that the PAS reaction is due to a lipid material which has no carbohydrate component, but in which 1, 2-glycol groupings have resulted from the oxidation of ethylenic double bonds, either during life or during the preparation of the sections. The failure to sulphate the material of the intercellular walls, and the failure to produce carboxyl groups by prolonged chromic acid oxidation, are probably not positive evidence against the presence of a mucopolysaccharide, for it must be pointed out that these techniques succeeded with only part of the intracellular glycogen—the part which is more persistent than the rest, and which is the last to be removed by digestion with saliva or diastase. It is probable, therefore, that not all polysaccharides can be successfully demonstrated by these techniques, and the failure of these techniques with the intercellular walls of the vaginal epithelium may not exclude the presence of a mucopolysaccharide.

The intercellular walls of the intermediate and superficial zones of the vaginal epithelium in these two fetuses consist, therefore, of a lipoprotein complex, in which the protein contains sulphydryl and/or disulphide groups. In addition, it is likely that a neutral mucopolysaccharide is also present.

SUMMARY

A honeycomb arrangement of 'intercellular walls' is described in the vaginal epithelium of two late human fetuses. The intercellular walls give positive reactions with the PAS, PAAS and PFAS techniques, are sudanophilic, and show a positive

autoxidation-Schiff reaction. The PAAS, PFAS and autoxidation-Schiff reactions are due to an unsaturated lipid, while the PAS reaction probably indicates the presence of a neutral mucopolysaccharide. Protein-bound sulphhydryl and disulphide groups, demonstrable by the ferric ferricyanide technique, are also present. It is concluded that the intercellular walls consist of a lipo-protein, which is probably associated with a mucopolysaccharide.

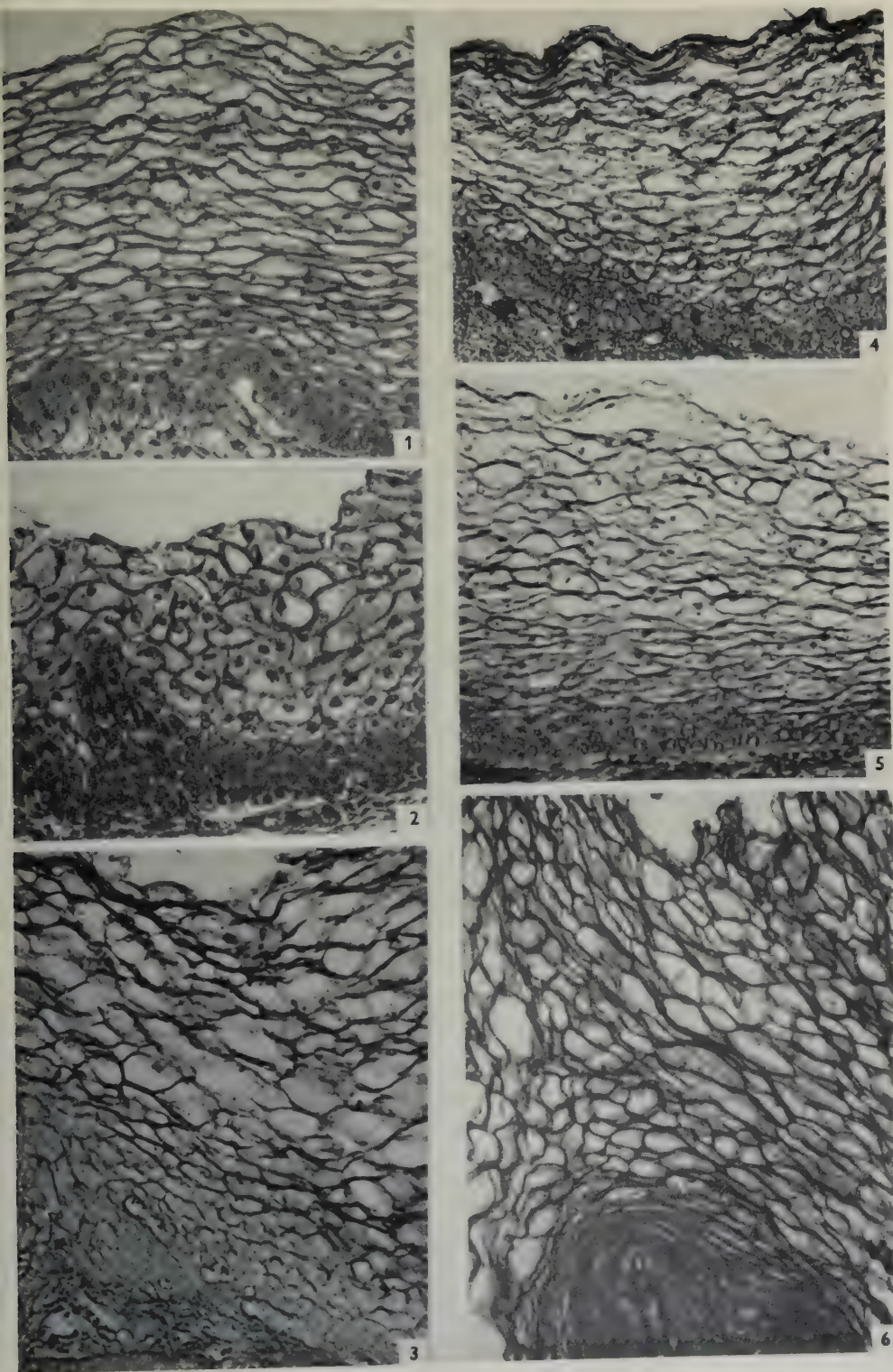
I wish to express my thanks to Mr A. Cain, A.R.P.S., who has produced the photomicrographs shown in Pl. 1.

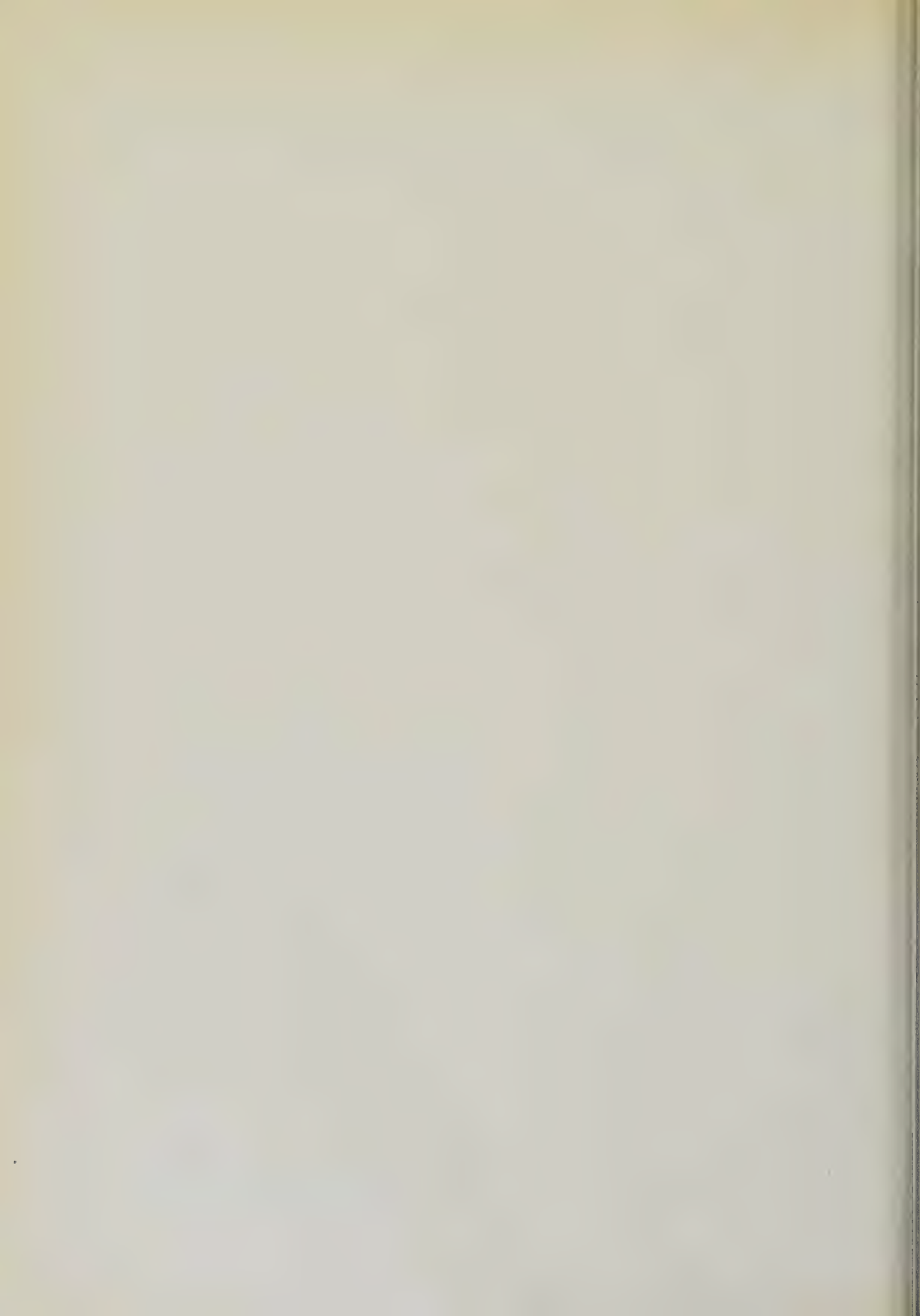
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EXPLANATION OF PLATE

- Fig. 1. Vaginal epithelium of 180 mm. foetus. Trichrome stain. ($\times 250$.)
- Fig. 2. Vaginal epithelium of 375 mm. foetus. Trichrome stain. ($\times 250$.)
- Fig. 3. Vaginal epithelium of 180 mm. foetus. PAS technique, after salivary digestion. In addition to the strong reaction of the intercellular walls, some cytoplasmic material gives a weaker reaction. ($\times 250$.)
- Fig. 4. Vaginal epithelium of 180 mm. foetus. Sudan black B. ($\times 250$.)
- Fig. 5. Vaginal epithelium of 180 mm. foetus. PFAS technique. ($\times 250$.)
- Fig. 6. Vaginal epithelium of 180 mm. foetus. Ferric ferricyanide technique, after treatment with Gram's iodine and sodium thioglycollate solution. ($\times 250$.)





OBSERVATIONS ON THE DEVELOPMENT OF THE SEMINAL VESICLES, PROSTATE AND BULBOURETHRAL GLANDS IN THE RAM

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INTRODUCTION

The literature relating to the embryonic development of the accessory genital organs is not voluminous and is concerned mainly with morphology, with only an occasional reference to the development of secretory activity. Johnson (1920) did distinguish mucous cells in the bulbourethral glands of a human embryo with a C.R. length of 14·7 cm., but did not indicate whether these discharged their secretion into the gland ducts. A reasonably exhaustive search of the literature has not revealed any reference to the embryonic development of the accessory genital glands of the ram. Particular interest attaches to the prostate in the latter species since it is exclusively disseminate in type, the gland tubules being located in the lamina propria of the pelvic urethra surrounded, more or less completely, by the striated urethral muscle and extending from about the level of the colliculus seminalis to the bulbourethral glands. Moreover, the secretory epithelium of this gland is closely similar to, and perhaps identical with, that of the bulbourethral glands and thus ram prostate resembles the glands which are occasionally found in the membranous urethra of human embryos (Lichtenberg, 1906; Johnson, 1920), and which Lichtenberg called accessory Cowper's glands. Yao & Eaton (1954) described the post-natal development of the accessory genital glands of the goat but did not describe their embryonic development.

MATERIAL AND METHODS

The embryonic material described hereafter was obtained from sheep of various breeds, mainly Blackface crosses, found to be pregnant on slaughter at the local abattoir. Collections were made at intervals during the 4-month period November to February inclusive. When recognizable on naked eye examination the seminal vesicles and bulbourethral glands were dissected out before fixation, and in such cases portions of the pelvic urethra, including the prostate, were also taken. In the case of younger embryos the entire pelvic urethra was removed and blocked in paraffin wax, serial sections then being cut, or serial sections of entire embryos were prepared. The material described was derived from seventeen embryos ranging from a C.R. length of 3·7 cm. (approximately 36 days—Winters and Feuffel, 1936) to 49 cm. (approximately full term). Adult material used for comparison with embryonic material was obtained from normal and castrated adults slaughtered at the abattoir. Material was fixed in corrosive formol or buffered neutral formol.

Sections from all blocks were routinely stained with haematoxylin and eosin, trichrome stain (Gomori, 1950), PAS (Gomori, 1953) and by PAS after incubation

in saliva for 15 min. at 37° C. Sections of material obtained from bulbourethral glands and prostate were also stained with Southgate's mucicarmine, alcian blue (Steedman, 1950), toluidine blue (pH 3.5-7), Sudan black and PAS after incubation in a mixture of equal parts methanol/chloroform at 37° C. for 1 hr.

RESULTS

Seminal vesicles

In the 5.4 cm. embryo the seminal vesicles were represented by a slight lateral extension of the ductus deferens close to the union of the latter with the urethra. At 7 cm. this extension had become a short tube directed dorsolaterally and then forwards (Pl. 1, fig. 1). Thus almost from its first appearance the tube which develops into the seminal vesicle is provided with a lumen. In all older embryos branching had taken place to an extent that varied with the age of the specimen, and in all cases tubules appeared to develop a lumen very shortly after formation, and hence at all developmental stages most of the tubes in the gland were canalized. In embryos measuring up to approximately 20 cm. the mesenchyme surrounding individual ducts was condensed with concentrically arranged tissue elements and these comparatively dense areas were separated from similar areas by a loose mesenchyme (Pl. 1, fig. 2). Examination of older specimens indicated that these ducts were lobular ducts and that these branch within this mesenchymal concentration to form a lobule. Expansion of the lobule during development resulted in the disappearance of the loose tissue and the distinction between lobules was then less apparent. Ultimately, however, the lobules became surrounded by a thin layer of smooth muscle (Pl. 1, figs. 3, 6). The muscle fibres did not penetrate between individual tubules.

Recently formed tubules were found to be provided either with a simple epithelium or with a stratified epithelium usually consisting of two layers. In the latter case the nuclei were distributed throughout the thickness of the epithelium and were not concentrated at any particular level. In older tubules, however, the epithelium was simple columnar and the nuclei tended to be located near the luminal surface of the epithelium where they formed a closely packed layer leaving the basal part of the epithelium with few or no nuclei (Pl. 1, fig. 4). This feature was noted in embryos of 15 cm. C.R. length onwards, although the time of first appearance varied, and in younger embryos was restricted to occasional tubules. The comparatively anucleate basal part of the epithelium stained poorly or not at all in haematoxylin and eosin preparations. In older embryos the epithelium was two-layered with a second layer between the above-mentioned tall columnar cells and the basement membrane. The nuclei of the basal cells formed a more or less continuous layer but were less numerous than those of the superficial layer (Pl. 1, fig. 6). The appearance of the epithelium at this stage therefore was similar to that of the castrated adult (Aitken, 1955). In the foetal gland, however, the epithelium is taller than in the castrate. Thus in the 15.6 cm. embryo the epithelium ranged in height from 18 to 31 μ and height increased with age to reach 35 to 40 μ in the 49 cm. embryo, whereas in the castrated adult the epithelium ranged from 10 to 25 μ (Aitken, 1955). As in the castrate, however, the free surface of the epithelium in all foetal

specimens was regular and sharply defined, thus differing from the normal adult ram in which the surface is very irregular with tongues of cytoplasm projecting from the free surface (Aitken, 1955).

The glandular epithelium in all embryos contained PAS positive granules which were also positive with Best's carmine and which were destroyed by pretreatment with saliva.

Bulbourethral glands

In the 3.7 cm. embryo the urethra in transverse section was in the form of a cross with four lateral extensions. Two of these were directed towards a mesenchymal condensation, forming a lateral extension of the urethral wall and these two were capped by solid buds of cells representing the anlagen of the bulbourethral glands. In the 5.4 and 6.25 cm. embryos the bulbourethral gland primordia appeared as evaginations which had extended to reach the above-mentioned mesenchymal concentration and a single branch had been formed on one side (Pl. 1, fig. 5). The gland primordium at this stage consisted mainly of a solid cord of cells, a lumen being present only in the proximal part near its attachment to the urethra. In the 8.8 cm. embryo the branches of the original diverticula were more numerous though still solid, but in the 10.5 cm. embryo many of the ducts were provided with a lumen although this was comparatively narrow. At this stage in development the tubules were sharply outlined by a thin layer of concentrically arranged fibroblasts and were lined by a stratified epithelium. Many of the gland ducts were in close contact with one another (Pl. 2, fig. 7). This was not a feature of the 19.4 cm. embryo in which the tubules were more widely separated by connective tissue (Pl. 2, fig. 8). Here also the majority of the tubules had a lumen and this was more prominent than in the younger embryos. Some of the tubules of this embryo were lined by a simple epithelium, whilst in others the epithelium consisted of two or more layers and in most cells the cytoplasm was comparatively scanty. Some cells, however, had a more abundant cytoplasm which stained feebly or not at all in haematoxylin and eosin preparations, and some of these gave staining reactions indicative of mucin. Similar cells were found in the lumen of occasional tubules where they frequently exhibited signs of degeneration. Other tubules contained cell debris.

In all older embryos of this series the individual ducts and alveoli of bulbourethral glands were structurally similar to those of the adult, the embryonic gland differing mainly in size and in the relative amounts of specific gland tissue and connective tissue, the latter being more abundant in the embryo (Pl. 2, figs. 9, 10). In such glands the larger ducts were lined by a transitional epithelium identical with that of the urethra, and these opened into ducts lined by a two-layered epithelium which in turn gave off tubules lined by a simple epithelium. In the latter the blind end of the tubule was frequently lined by tall pyramidal cells with flattened nuclei placed in contact with the basement membrane. At the periphery of the gland, however, there were still some lumenless cell cords.

In all embryos up to a C.R. length of 10.5 cm. the epithelial cells contained numerous globules which gave a positive reaction with the PAS staining method, but which were destroyed by incubation in saliva and which therefore were presumed to be glycogen. Glycogen was absent from the duct epithelium of all older

embryos of the series except in ducts lined with a transitional epithelium where, as in the urethral epithelium, glycogen droplets were abundant. In the 17 cm. embryo, however, the epithelium of some tubules contained a substance which gave a positive reaction with PAS even after incubation in saliva, and was positive with Southgate's mucicarmine and alcian blue and metachromatic with toluidine blue at pH 3.5 (Pl. 2, fig. 11). A similar material was present in all older embryos in which, however, it was much more widely distributed and occurred not only within the cell cytoplasm but also free in the lumen of many ducts.

In the adult gland a similar material was extensively distributed, occurring in all the epithelial cells lining the tubules and alveoli. This material was present in greatest abundance in the large pyramidal cells lining the terminal alveoli, and an amorphous material giving similar staining reactions was found in the lumen of most ducts. Mixed with the latter substance, however, there frequently occurred globules or masses of a material which was readily distinguished from the amorphous substance by the fact that in sections stained by the Gomori trichrome method it was coloured bright red, whereas the amorphous material stained a light green (Pl. 2, fig. 12). Not infrequently in such preparations the globules were surrounded by a very thin layer of green stained substance. These globules were PAS positive even after incubation in saliva or in a mixture of equal parts methanol and chloroform, but were negative with Sudan black B and were not metachromatic with toluidine blue at pH ranging from 3.5 to 7. The number of these globules varied widely in the adult gland, and in any one specimen they varied widely in size. The source of this material was not ascertained, although in one specimen the epithelial cell cytoplasm did appear to contain some minute droplets of similar staining reaction. These were, however, at the limit of resolution. Large droplets comparable to those found in the gland ducts were not found in the epithelial cells. This material was completely absent from the foetal glands examined.

Prostate

The entirely disseminate prostate of the ram was found to consist of numerous compound tubular glands located in the lamina propria of the pelvic urethra and extending from approximately the point of entry of the ducts of the seminal vesicles to the level of the bulbourethral glands. The amount of glandular tissue varied at different levels and when particularly abundant formed an almost continuous mass in which it was difficult to distinguish individual lobules; elsewhere the lobules were separated by septa of dense connective tissue and lobulation was then distinct. The gland tissue sometimes completely encircled the urethra, but where less abundant was confined to the vicinity of the urethral crest. Where glandular tissue was absent the lamina propria contained numerous, large, thin-walled veins. Externally the lamina propria was encircled by the striated urethral muscle, internal to which at certain urethral levels there was an incomplete layer of smooth muscle fibres. This was particularly prominent towards the bladder where the fibres were arranged for the most part longitudinally, but was frequently absent from more caudal levels or, if present, represented by isolated groups of more or less circularly arranged fibres. Only occasionally at upper levels of the urethra did this smooth muscular tissue

penetrate between the gland lobules. Occasionally, however, the glandular tissue penetrated into the striated urethral muscle, sometimes extending almost throughout its thickness.

There was no sign of development of the prostate in the 3.7 and 5.4 cm. embryos, this gland apparently lagging slightly behind the bulbourethral in its formation. This lag was also indicated by the fact that the prostatic tubules developed a lumen later than those of the bulbourethral glands, a difference which was still noticeable in the 17 cm. embryo in which tubules with a lumen were much less frequent in the prostate than in the bulbourethral gland from the same embryo. In the 19.4 cm. embryo and in later embryos, however, these differences were no longer recognizable.

In the 6.25 cm. embryo the prostate took the form of a few solid buds of cells on the urethral epithelium (Pl. 3, fig. 13). In the older embryos solid cords of cells extended into the urethral lamina propria which even in the 6.25 cm. embryo contained numerous dilated sinusoidal blood vessels, the cavernous character of this tissue apparently developing at a very early stage. In the 10.5 cm. embryo some of the tubules had become canalized, but the majority were lumenless.

Apart from the above-mentioned lag the prostate and bulbourethral gland were similar in development and were also similar structurally. Thus in both, the secretory tubules were lined by a morphologically identical, simple epithelium and in prostatic, as in bulbourethral, the blind ends of the tubules were frequently lined by large pyramidal cells with flattened, basally located nuclei (Pl. 3, fig. 14). The latter were present in the prostate of the 21.9 cm. embryo and in all older embryos. The excretory ducts were lined by a two-layered epithelium which was replaced by a transitional epithelium near the point of entry into the urethra.

In the earlier stages of its development the prostatic epithelium like that of the bulbourethral gland contained glycogen. In older embryos, however, glycogen was absent except from the ducts lined by a transitional epithelium and as in the bulbourethrals the disappearance of glycogen was associated with the appearance in the cells and in the lumen of secretory granules which were PAS positive even after treatment with saliva and which were positive with Southgate's mucicarmine and alcian blue, and metachromatic with toluidine blue at pH 3.5. In the prostate, however, the change was found to take place at a later stage than in the bulbourethral gland. Thus glycogen was still present in the 17 cm. embryo, but had been replaced by mucus in the 20.2 cm. embryo (Pl. 3, fig. 15).

In the 49 cm. embryo the appearance of the prostate approached that of the normal adult, but the gland tubules were not so numerous and the intertubular connective tissue correspondingly more abundant (compare Pl. 3, figs. 16, 17). The gland at this stage was similar in its degree of development to that of the castrated adult (Pl. 3, fig. 18).

The primordium of the urethral muscle was present in very early embryos, and internal to this a thin layer of smooth muscle could be distinguished from the 20.2 cm. stage onwards. This muscle did not extend between the gland tubules at any stage in development, although in occasional embryos, as in the adult, a few gland tubules sometimes extended into the striated urethral muscle.

As in the embryonic glands the secretory cells of the adult gland contained a metachromatic, PAS positive substance. This was most abundant in the large

pyramidal cells lining the blind ends of the gland tubules, but occurred at all levels except in the larger excretory ducts lined by transitional epithelium. The pyramidal cells were usually so packed with secretion that individual granules could not be distinguished. In other cells, however, the granules were less numerous and usually discrete. The amount of material in individual cells varied widely, however, and although in a given tubule all of the cells in a transverse section might contain similar amounts of secretory material, occasionally single cells or small groups of cells containing an abundance of secretory granules were interspersed with cells containing comparatively few granules. The prostatic PAS positive material was not destroyed on incubation in saliva or in methanol chloroform and was not stained by Sudan black B. Most of the granules also appeared to react with mucicarmine and alcian blue, but although these reactions were generally co-extensive with the PAS reaction this was not always so. This may reflect a less intense reaction where secretory granules are less numerous, but the metachromasia with toluidine blue at pH 3.5 was distinctly less extensive than the PAS reaction. Furthermore, on staining with Masson's trichrome stain some cells were found to contain golden-brown granules whilst others contained greenish mucin granules, and there was at least some degree of correspondence between the distribution of the latter and metachromasia.

The prostate glands derived from castrated animals appeared to contain less glandular tissue than the normal prostate, and intertubular connective tissue was correspondingly more abundant. In many cases, however, the difference was slight although castration in every case had been performed some months prior to slaughter. In some of these glands secretory activity appeared to equal that of the normal animal whilst others appeared to be less active. The staining reactions were also generally similar in castrated and normal animals, but in some of the castrated animals golden-brown granules were not noted in trichrome preparations. It is difficult to estimate accurately whether there is a reduction in cell height after castration since fairly marked variations occur in any one gland, but if a reduction does follow castration it is small and not readily discerned. Cell heights are also similar in near term embryos and normal adult rams.

DISCUSSION

Watson (1918) described the development of the seminal vesicles in man and noted their first appearance in an embryo of 8 cm. c.r. length in which they appeared as a small lateral evagination of the Wolffian duct. A similar but less prominent evagination of these ducts was noticed in a sheep embryo of 5.4 cm. By 7 cm. this had become a tube directed at first laterally and then forwards and in slightly older embryos this tube had begun to branch to form the lobular ducts. These tubules appeared to develop a lumen almost as soon as formed, whereas Yao & Eaton (1954) found that most of the tubules of the seminal vesicles of goats were lumenless at 30 days after birth. This difference cannot be explained on the basis of increasing height of the epithelial cells since in the 49 cm. embryo the epithelium ranged from 35 to 40 μ , whereas Yao & Eaton found the corresponding cells in the goat to average 36 μ . At this stage of foetal development, the epithelium of the sheep

seminal vesicle resembles that of the 30-day goat (Yao & Eaton, 1954) and presents features which are characteristic of the castrated adult (Aitken, 1955). Thus the free surface of the glandular epithelium is sharply outlined and lacks the protoplasmic protrusions characteristic of the normal adult; the epithelium is two-layered and is filled with glycogen droplets. Again the proportion of glandular tissue to connective tissue is approximately the same in the late foetus and castrated adult. Nevertheless, foetal glands show evidence of androgenic stimulation, the epithelial cells being taller than in the castrated animal, although still slightly smaller than in the normal adult.

Eggerth (1915) described the development of lateral ridges on the wall of the urogenital sinus of the human embryo and depicted the development of the bulbourethral glands from a solid knot of cells which appeared on the anterior end of the middle ridge at 3.0 cm. Müller (1892) described similar ridges on the urethral wall of the early bovine embryo. These soon became hollow when the urethral canal in transverse section had the form of a cross. Müller found that the bulbourethral glands first appeared at 6 cm. as a solid bud of cells on the crest of two of these ridges. The bulbourethral glands of the sheep thus resemble those of the bull in their early development, but are most closely related to the human in the time of their first appearance. Lowsley (1912) found no sign of prostatic development in the 5 cm. human embryo but observed very considerable development at 7.5 cm. Johnson (1920) found evidence of development of prostate and bulbourethral glands in a human embryo of 5.5 cm.

In the ram the prostate appears to lag slightly behind the bulbourethral glands in development, not only with regard to the time of first appearance but also with regard to the time of onset of secretory activity. Apart from this, however, these glands are very closely similar in the foetus both from the histological and histochemical point of view. In both, the individual gland tubules acquire adult appearance at a very early stage in development and at the same time acquire the ability to secrete. The secretion found in the tubules of the adult bulbourethral gland, however, contains substances not found in the secretion of the embryonic gland and not found in the prostate of the adult. Both the embryonic and adult bulbourethral glands secrete a sulphated mucopolysaccharide (giving a positive PAS reaction even after treatment with saliva and exhibiting metachromasia with toluidine blue). In addition, however, the secretion of the adult gland contains a non-metachromatic substance which gives a positive PAS reaction which is unaffected by incubation in saliva. This substance is not removed on incubation in methanol/chloroform and is Sudan black negative and therefore does not appear to be glycolipid. Presumably it is a form of mucoprotein. In both embryonic and adult prostate the cells contain a PAS positive substance which also exhibits metachromasia with toluidine blue and a similar material may be found in the gland ducts. In sections of adult prostate stained by trichrome methods, however, the granules in some of the secretory cells are coloured golden brown, a reaction not noted in any of the cells of the embryonic prostate and also absent from the cells of the bulbourethral gland.

Johnson (1920) found tubulo-alveolar glands which were histologically identical with Cowper's glands in the mucosa of the membranous urethra of occasional human embryos. Lichtenberg (1906) found similar glands in the bulbous part of the urethra in occasional human embryos and because they were closely related topographically

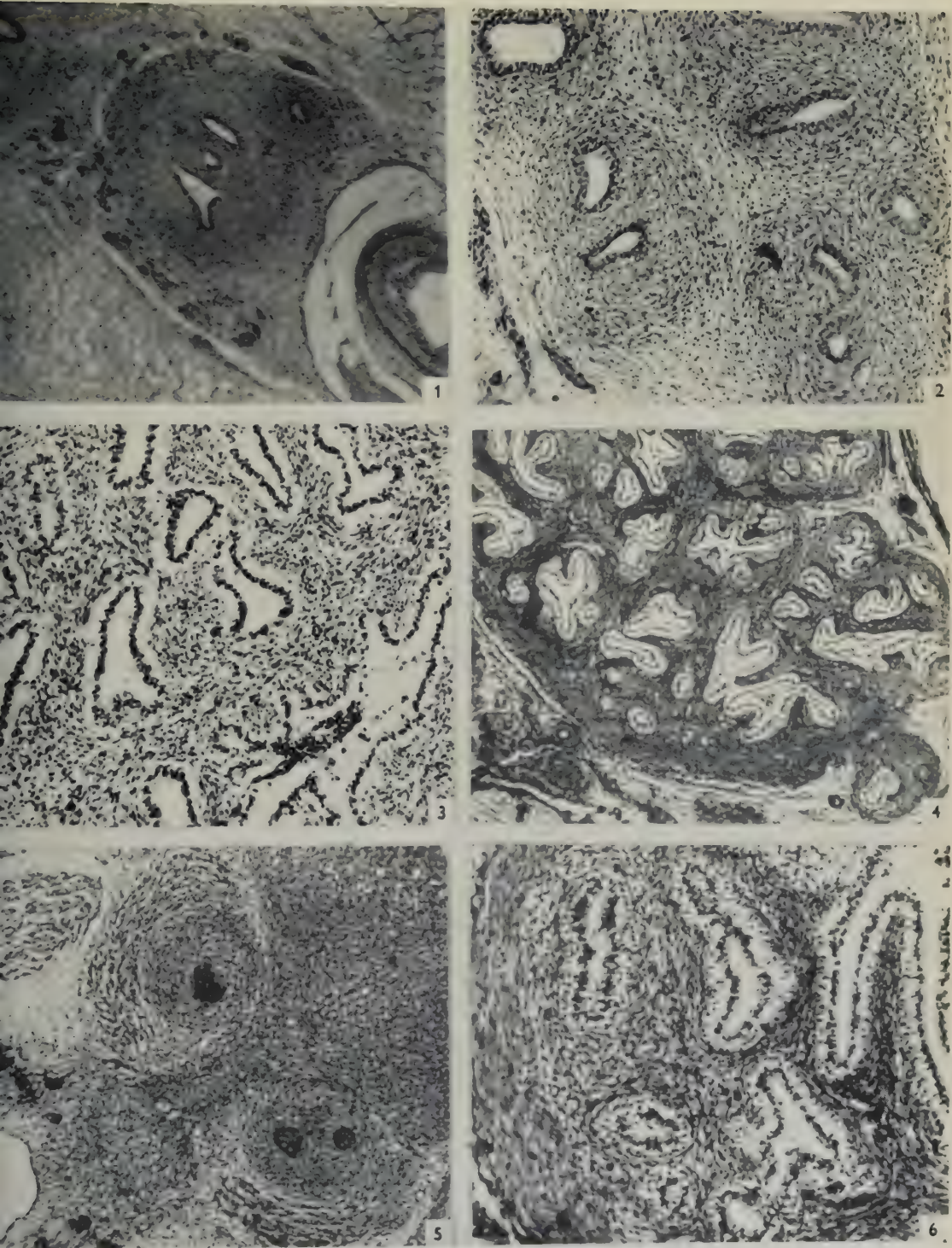
to the ducts of the bulbourethral glands and were histologically similar to the latter, called them accessory Cowper's glands. The tubulo-alveolar glands, which together form the disseminate prostate of the ram, are a constant feature in this species and are very much more numerous than the corresponding glands in the human. Like the accessory Cowper's glands of Lichtenberg, however, they are similar, developmentally and structurally, to the bulbourethral glands, although they may yet prove to have functional differences. In this connexion it is worth noting that in human embryos mucous cells have appeared in the bulbourethral glands by 14.7 cm. (Johnson, 1920), whereas there is no evidence of secretion by the prostate at this stage. Arcadi (1952) noted mucus secretion by the prostatic epithelium of the dog at 8–12 months, but found no evidence of secretion at 1 day post-partum. Huggins, Masina, Eichelberger & Wharton (1939) also noted the absence of secretion in the immature dog. In sheep, on the other hand, secretory activity by the prostate begins in the early embryo only shortly after the onset of secretory activity by the bulbourethral gland. A further feature of interest in this connexion is the comparatively slight structural differences between the prostate in the normal ram and in the castrate, suggesting that the influence of androgens on prostatic secretion may be less important in relation to the disseminate prostate of the sheep than in other species. Yao & Eaton (1954) also found secretion in the lumen of prostatic tubules of all their specimens (ranging from 30 to 210 days post-partum). In their younger specimens, however, the epithelial nuclei appear to have been located in the luminal half of the cell, whereas in the foetal sheep the epithelial nuclei in the prostate are located in the basal half of the cell from a very early stage in development and in the alveoli are frequently pressed against the basement membrane and flattened.

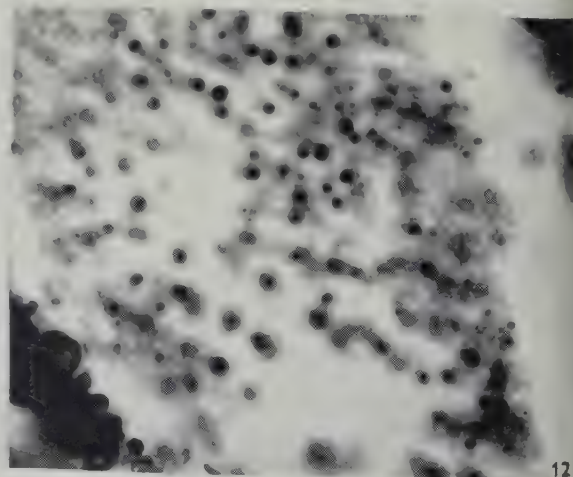
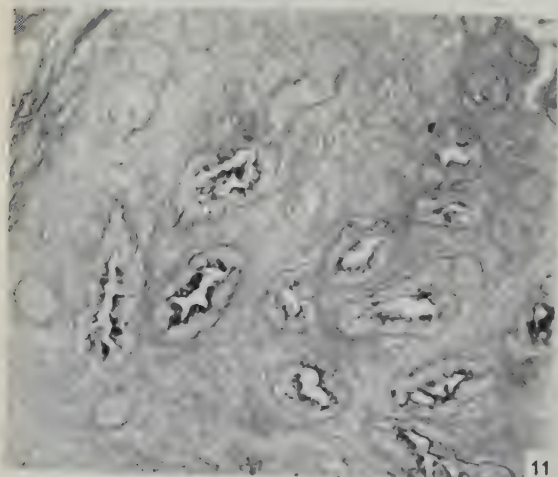
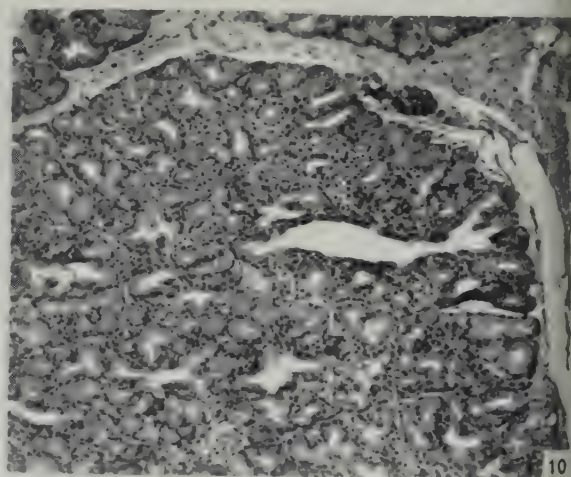
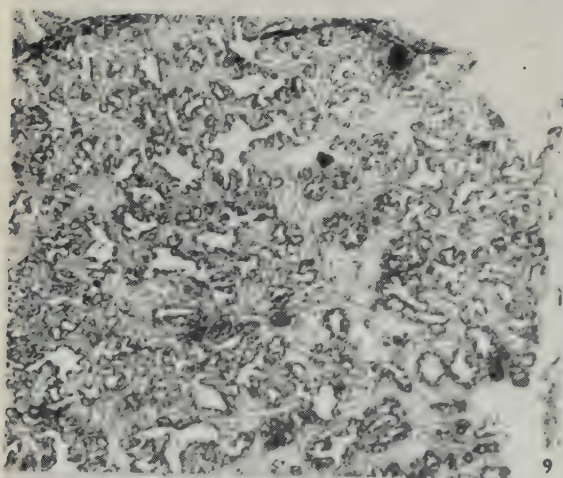
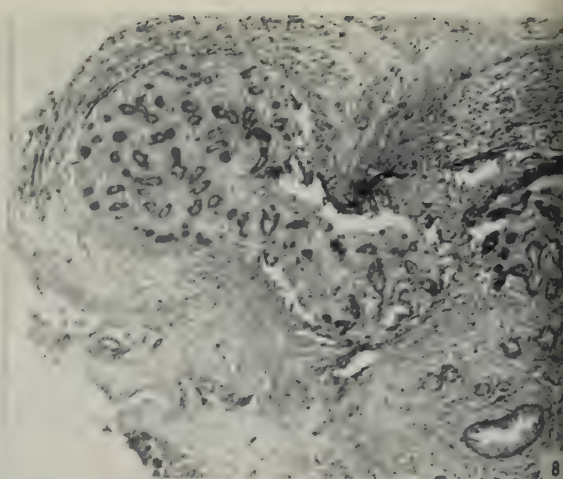
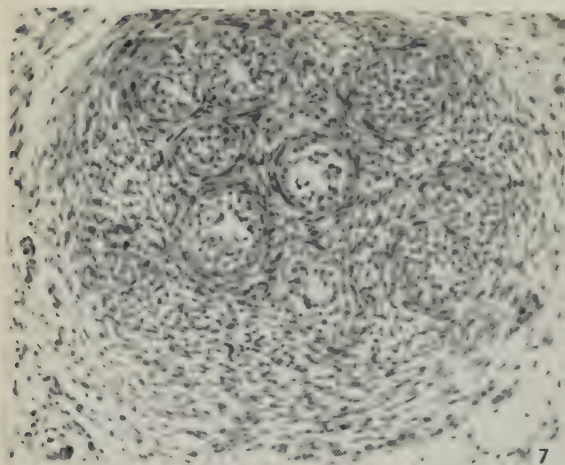
SUMMARY

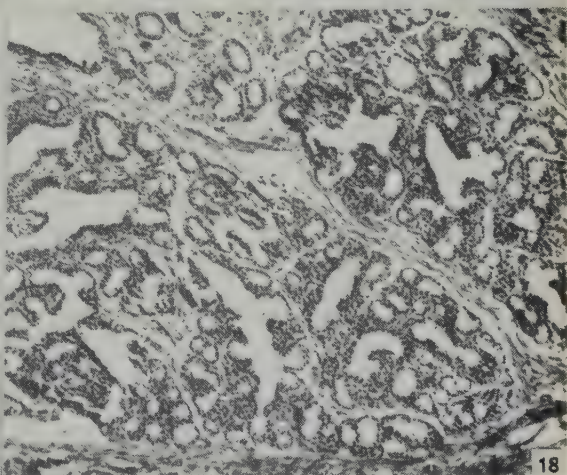
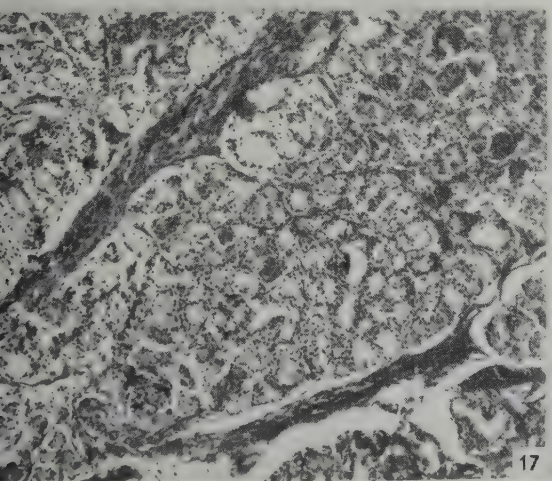
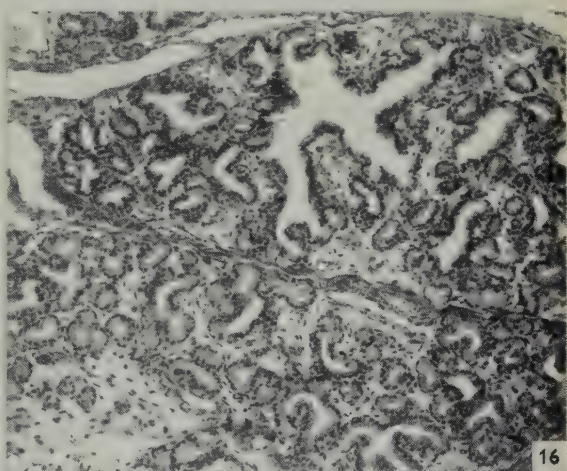
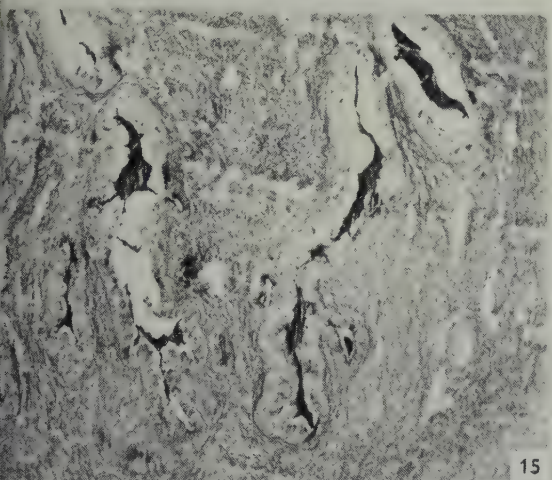
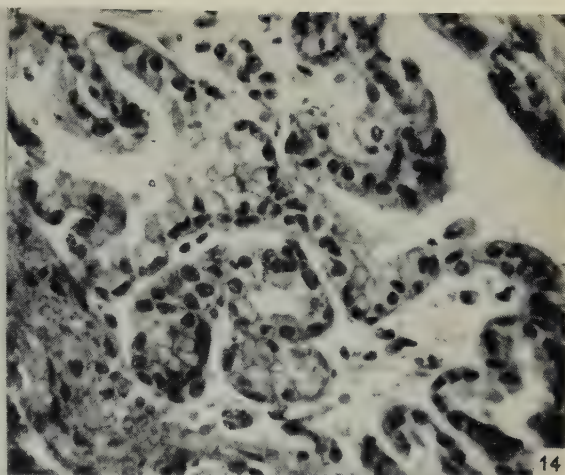
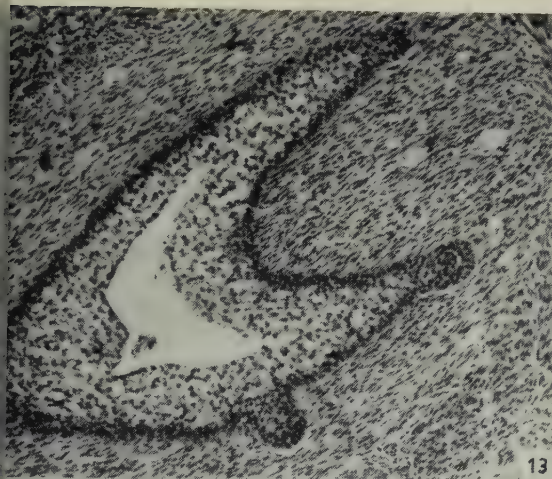
1. The development of the seminal vesicles, prostate and bulbourethral glands in the sheep has been described.
2. It has been shown that the seminal vesicle in the late-term foetus is morphologically similar to that of the castrated adult, although the influence of testicular androgens in the former is suggested by the greater height of the secretory cells.
3. The prostate and bulbourethral glands in this species are developmentally, morphologically and functionally similar, but there may be slight differences in the composition of the secretion in the adult.
4. Both prostate and bulbourethral glands in the sheep are functionally active in embryos of between 17 and 20 cm. C.R. length and the individual gland tubules are then morphologically similar to those of the adult.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Section through terminal part of ductus deferens. Seminal vesicle appears as a simple tube-like diverticulum. C.R. length 7 cm. $\times 40$.
- Fig. 2. To show appearance of seminal vesicle in 19.6 cm. embryo. $\times 100$.
- Fig. 3. Seminal vesicle from 49 cm. embryo. Perilobular muscular tissue can be distinguished. $\times 35$.
- Fig. 4. Seminal vesicle from 37.9 cm. embryo. Epithelium consists of a single layer of columnar cells with all nuclei close to the luminal surface. $\times 100$.
- Fig. 5. Seminal vesicle from 49 cm. embryo. To show two-layered epithelium characteristic of this stage. $\times 100$.
- Fig. 6. Bulbourethral gland from 6.25 cm. embryo. The gland appears as a solid cord of epithelial cells. Branching is visible on one side only. $\times 40$.

PLATE 2

- Fig. 7. Bulbourethral gland from 10.5 cm. embryo. Glandular epithelium is stratified and gland tubules are surrounded by concentrically arranged fibroblasts. $\times 140$.
- Fig. 8. Bulbourethral gland from 19.4 cm. embryo. $\times 35$.
- Fig. 9. Bulbourethral gland from 49 cm. embryo. $\times 35$.
- Fig. 10. Bulbourethral gland from normal adult. Glandular tissue is more abundant relative to connective tissue when compared with the full-term embryo. $\times 100$.
- Fig. 11. Bulbourethral gland from 17 cm. embryo. Stained by the PAS method. $\times 100$.
- Fig. 12. Bulbourethral gland from normal adult. To show globules in secretion. In trichrome preparations these are coloured red and some have a thin sheath of greenish material, the outline of which is visible in the photograph. Gomori trichrome. $\times 1000$.

PLATE 3

- Fig. 13. T.S. urethra from 6.25 cm. embryo. Prostate consists of solid buds of epithelial cells. $\times 100$.
- Fig. 14. Prostate from 49 cm. embryo. $\times 400$.
- Fig. 15. Prostate from 20.2 cm. embryo. Secreted material can be distinguished in gland ducts. Alcian blue. $\times 100$.
- Fig. 16. Prostate from 49 cm. embryo. To show the ratio of glandular tissue to connective tissue at this stage. Glandular epithelium is similar to that of the adult. $\times 100$.
- Fig. 17. Prostate from normal adult. $\times 100$.
- Fig. 18. Prostate from yearling castrate. $\times 100$.

RADIOLOGICAL STUDIES OF THE GROWTH OF THE PITUITARY FOSSA IN MAN

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It has been suggested by several workers (Maier, 1936; Davenport & Renfroe, 1940; Francis, 1948) that the pituitary fossa undergoes a growth spurt at about the time of puberty. Reviewing this, and other research of a similar nature, Tanner (1955, p. 116) writes 'the dimensions of the sella turcica show a very considerable growth spurt at adolescence which sets this structure aside from the rest of the skull in its growth curve'; yet, although this assertion is of interest and importance, no study made to date, considered by itself, adequately supports it. Thus, we have taken advantage of an opportunity to measure serial X-rays of the skull of a group of children, taken at regular intervals throughout their growing life, in an attempt to investigate the matter further.

THE LITERATURE

The literature concerned with measuring the pituitary fossa from the lateral X-ray of the skull of healthy persons is extensive and confused. Most of the publications on the subject can be divided into two broad groups: first, those which seek to define the normal size range of the fossa and so to facilitate the detection of the pathological in clinical medicine (Enfield, 1922; Camp, 1924; Haas, 1925; Chaumet & Grigoratos, 1927; Büchner, 1952; Lorenz, 1949; Acheson, 1956; Bergerhoff, 1956; etc.); and secondly, those which are primarily concerned with the growth of the fossa (Gordon & Bell, 1921; Le Coulm, 1923; Hotz, 1928; Steiert, 1928; Kovács, 1934; Francis, 1948; Acheson, 1954; Silverman, 1957; etc.). Some authors (Gordon & Bell, 1921, 1925, 1936; Le Coulm, 1923; Rokhline, 1935; Francis, 1948), however, believe that the length and depth of the fossa are adequate indices of fossa size, whereas others have thought it necessary to consider the area of the sagittal section. Many techniques have been used to make an estimate of area, ranging from the relatively simple, such as product of length and depth (Royster & Rodman, 1922) or planimetry (Royster & Moriarty, 1930; Davenport & Renfroe, 1940; Silverman, 1957; etc.) to more complex methods such as either tracing the outline of the sella from the X-ray film on to a transparent sheet, superimposing finely calibrated graph paper and counting the squares contained by the shadow of the fossa (Haas, 1925, 1954), or tracing the fossa on paper of standard thickness, cutting out the image thus obtained and weighing it (Burrows, Cave & Parbury, 1943).

The details of techniques for measuring length and depth also differ widely in the various studies (for fuller review see Acheson, 1954; Silverman, 1957), but the basic principle is always to draw a line in the sagittal plane of the fossa which is the 'length', and to take the 'depth' as the distance from the introitus to a point on the floor of the fossa. Thus, exact definition is only necessary at a few points on the

X-ray. On the other hand, with the exception of the first-listed, all the methods of estimating area have the *a priori* disadvantage that the exact definition of the entire contour of the fossa is necessary. A procedure of this sort is liable to error because a contour, such as that of the sella, which is some distance from the film when the X-ray is being taken, usually tends to be slightly blurred. Often, moreover, the normal fossa appears to have three contours (Bokelmann, 1934; Haas, 1934) the radiographic relations of which can be disturbed by minimal rotation of the head. Calculating the product of length and depth is not a satisfactory substitute for the more meticulous methods of estimating area, and it correlates very poorly with them (Royster & Moriarty, 1930; Burrows, *et al.* 1943; Silverman, 1957). Unless, therefore, there is some good reason for studying area, the simple dimensions of length and depth should perhaps be accepted as the most reliable index of fossa size when the estimate is made from simple radiograms.

Interest in fossa area has, nevertheless, persisted and it seems that, in clinical research at least, much of the work along these lines has been based on a misconception. In various reports (e.g. Camp, 1923, 1924; Cardillo & Bossi, 1941; Namiki, Kobayashi & Mashima, 1938; Karlas, 1948) it has been shown that the area of the sagittal section of the fossa on the X-ray correlates more closely with the volume of the *fossa* than do length or depth. From this it has been presumed that the area of the fossa must also be a reliable indicator of the size of the pituitary *gland* (Haas, 1925; Schinz, 1928; Silverman, 1957), and even of the activity of the gland (Schinz, 1928, 1952; Haas, 1929; Lisser & Escamilla, 1957; Silverman, 1957). All the evidence suggests that these presumptions are without basis, for no consistent relationship has been found between the size of the pituitary gland and its fossa (Bokelmann, 1934; Ottaviani, 1938; Marx, Hesse & Neumann, 1947; Busch, 1951; Dill, 1953), nor, with the possible exception of some pituitary glands which have undergone pathological enlargement, does any relationship exist between size or area of fossa and *activity* of the gland (Gordon & Bell, 1925, 1936; Perona, 1938; Nobécourt & Hagenau, 1939; Marx *et al.* 1947). It is, however, only fair to add here that in some modern texts (e.g. Jupe & Northfield, 1956; Pendergrass, Schaeffer & Hodes, 1956) it is plainly indicated that fossa size should not be looked upon as an index of gland function.

In the present paper, therefore, we shall confine our attention to the length and depth of the fossa.

MATERIAL AND METHODS

Our observations were made from lateral X-rays of the skull in the Bolton collection at Western Reserve University, Cleveland, Ohio. The Bolton collection comprises serial lateral and antero-posterior X-rays of the skull of a large group of children taken at regular intervals throughout their growing years. In some cases these records, which have been compiled in a study of the growth of the face and jaw, are complete from early infancy to the age of 20 years. By using the Broadbent-Bolton cephalometer (Broadbent, 1931), the position of the child is rigidly standardized so that the successive X-ray films are superimposable, and the tube-film distance (30 ft.) is such that the size of the fossa on the film 'is not distorted, nor altered to any appreciable degree' (Francis, 1948). In addition to these X-rays, each child

had his height and weight measured at every visit and notes were made of his general health. For the present purpose 40 children (22 boys and 18 girls) were chosen whose dento-facial development was good, and whose records were the most complete; there was a total of 688 attendances.

All measurements were made by one of us (R. M. A.) with a scissors-type caliper, incorporating a magnified scale which permitted readings to 0.1 mm. (Tanner, 1951*a*). The length of the fossa was taken as the distance from the tuberculum sellae to the top of the dorsum sellae, two points which with experience can be identified fairly reliably on the lateral X-ray of most individuals (Fischgold & Metzger, 1953; Acheson, 1956; Silverman, 1957) and the depth as the shortest distance from this line to the lowest point of the sella. Since we had been asked not to mark the X-rays, a straight line was engraved on a thin piece of transparent plastic material which was placed between the X-ray film and the illuminated screen in such a way that the line ran from the tuberculum to the dorsum sellae. The depth was then measured from the engraving to the appropriate point on the film. Sometimes a child had two lateral films taken at one visit, usually because it had moved; on 44 occasions (30 in boys and 14 in girls), however, the movement was confined to the jaw and in such cases the pituitary fossa was clearly defined. In this event both films were measured and the average of the two measurements used, so that a grand total of 732 X-rays was studied. Some, but not all, of these films were included in the series measured by Francis (1948).

RESULTS

Statistical analysis of data. The mean values for length and depth were calculated and these are shown, together with standard deviations, by age and sex in Table 1 and Figs. 1 and 2. There is no consistent tendency for the fossae of either sex to be longer or deeper than those of the other sex (Fig. 1), and such differences as exist are very slight. In contrast to this similarity between the mean values, the standard deviations in the boys are consistently slightly bigger for both length and depth than in the girls.

The curves in Fig. 1 suggest that there may be a slight increase in rate of growth in length and depth in girls aged 11 to 14, and in depth in boys in their 15th year. The mean increments in length and depth, which are shown in Table 2* and Figs. 3 and 4, lend support to this suggestion that there is a growth spurt at the time of puberty for depth in both sexes, but there is little evidence of any spurt for length. It is well known, however, that within either sex children mature at different rates and so experience their pre-adolescent growth spurt at different ages. Therefore, a genuine pre-adolescent growth spurt in the growth rate of the pituitary fossa could be masked by the treatment shown in Figs. 1 and 2.

To overcome this difficulty the mean increase in length and depth of the pituitary fossa during each year from the age of 5 years to maturity was calculated for each child; the mean annual increase in standing height over the same period was also

* The increments in Table 2 were calculated from the longitudinal part of the data only. Since the proportion of the data which, for any year was cross-sectional, was low, and since the value for r for serial measurements seldom fell below 0.9, it was not considered worth while to make a 'best estimate' of increments by the Patterson-Tanner procedure (Tanner, 1951*b*).

calculated. Preliminary study of the resulting data suggested that there was, as a rule, a growth spurt in the pituitary fossa, particularly in respect of depth, and that this tended to occur synchronously with the growth spurt in stature. Therefore, for every child the growth of the pituitary fossa was divided into three phases:

Phase 1. From age 5 years until the year before maximum growth in stature (i.e. the year before the adolescent growth spurt).

Phase 2. The year of the adolescent growth spurt in stature.

Phase 3. From the year after the adolescent growth spurt until the record ceased.

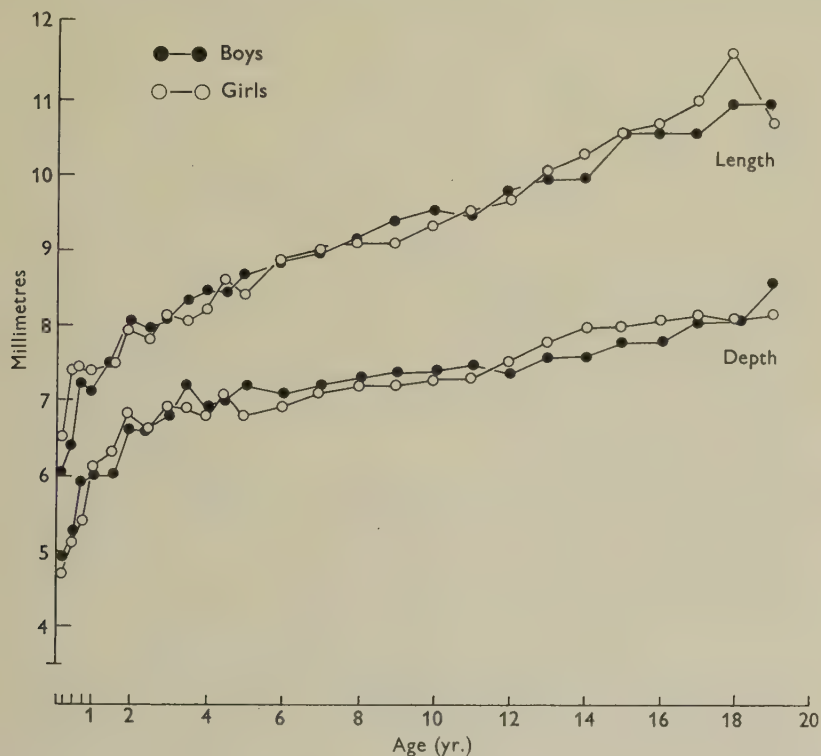


Fig. 1. Mean length and depth of the pituitary fossae of the children by age, sexes separately (see also Table 1).

Increments in length and depth of the fossa for the years constituting phase 1 were averaged for each child, as were those for years constituting phase 3. Then the means for the three phases were calculated for all the children for the sexes separately and in combination (Table 3). It will be seen that the mean growth in length and depth is greater during the year in which the pre-adolescent growth spurt occurs, than during the preceding or following periods. It also appears that growth continues more rapidly during the post-adolescent than during the pre-adolescent years. Statistical tests show that the differences between each of the three periods are significant in respect of depth, but not in respect of length (Table 4). A further point in Table 3, which is worthy of comment, is that the standard deviation of the mean is much bigger in both sexes in Phase 2, than in phases 1 or 3. The

most reasonable explanation for this is that the procedure of averaging the increments for each child in phases 1 and 3 reduced that part of the variance (the within-child variance) which was due to technical error; and that no such reduction occurred in phase 2, which, for each child, consisted of a single annual increment. This suggestion is borne out by the similarity between the standard deviation in phase 2 and those for each year shown in Table 2.

The fact that the pre-adolescent growth spurt was more pronounced in respect of depth than in respect of length raised the question of whether, when one of these

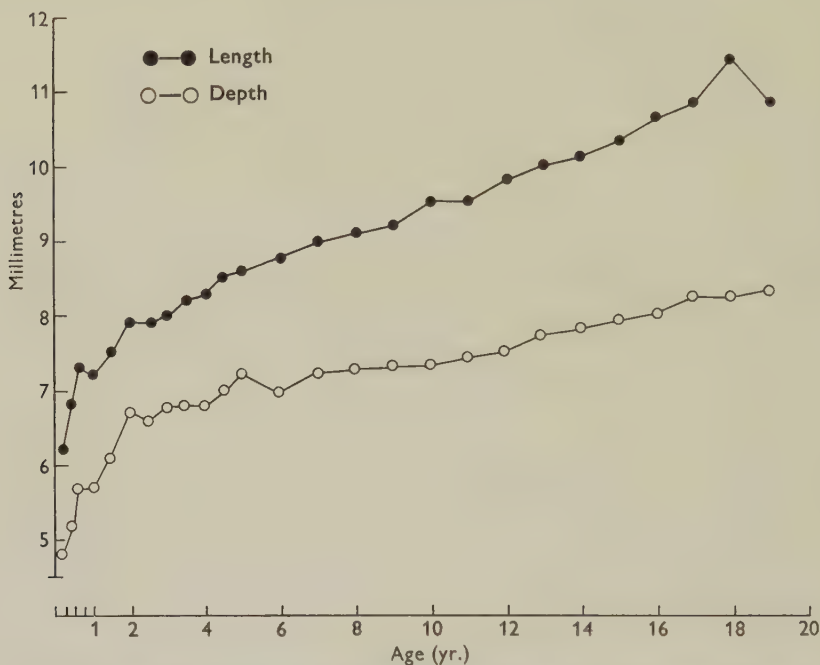


Fig. 2. Mean length and depth of the pituitary fossae of the children by age, sexes together (see also Table 1).

dimensions was plotted against the other on a logarithmic scale, the slope of the line at about the time of puberty would differ from the slope at other times. This did not, in fact, prove to be the case, because it was found that the curve, for the sexes combined, was rectilinear from the age of two years onwards (Fig. 5). However, during the first 2 years of life, although the curve was also rectilinear, the slope differed from that during the later period, indicating that growth in depth was occurring more quickly relative to growth in length than at any other time. This early period was not, of course, taken into account in the analysis shown in Tables 3 and 4.

Growth of the individual fossa. Statistical analysis is a method of demonstrating the general patterns to which the majority of cases conform. Nevertheless, just as there is a wide variety of shapes of adult fossae, so the growth pattern is also subject to considerable variation from one individual to another. In Fig. 6 are shown

Table 1. Mean length and depth of the pituitary fossa by age, sexes separately and together (millimetres)

Age (yrs)	Boys						Girls						Sexes together					
	Length			Depth			Length			Depth			Length			Depth		
	No.	Mean	S.D.	Mean	S.D.	No.	No.	Mean	S.D.	Mean	S.D.	No.	No.	Mean	S.D.	Mean	S.D.	No.
$\frac{1}{2}$	6	6.0	0.5	4.9	0.7	4	4	6.5	0.4	4.7	0.6	10	10	6.2	0.5	4.8	0.6	10
$\frac{1}{2}$	7	6.4	0.9	5.2	0.6	5	5	7.4	0.5	5.1	0.4	12	12	6.8	0.8	5.2	0.5	12
$\frac{1}{2}$	7	7.2	0.7	5.9	0.7	8	8	7.4	1.0	5.4	0.9	15	15	7.3	0.9	5.7	0.8	15
1	11	7.1	1.0	6.0	0.9	7	7	7.4	0.6	6.1	0.4	18	18	7.2	0.9	6.0	0.7	18
$1\frac{1}{2}$	12	7.5	1.2	6.0	0.9	7	7	7.5	0.8	6.3	0.5	19	19	7.5	1.1	6.1	0.8	19
2	11	8.0	1.1	6.6	1.0	8	8	7.9	0.8	6.8	0.8	19	19	7.9	1.0	6.7	0.9	19
$2\frac{1}{2}$	11	7.9	1.3	6.6	0.9	9	9	7.8	0.9	6.6	0.5	20	20	7.9	1.1	6.6	0.7	20
3	14	8.0	1.1	6.8	1.1	9	9	8.1	1.0	6.9	0.7	23	23	8.0	1.0	6.8	0.9	23
$3\frac{1}{2}$	13	8.3	1.8	7.2	0.9	8	8	8.0	0.8	6.9	0.5	21	21	8.2	1.0	6.8	0.8	21
4	17	8.4	1.5	6.9	1.2	10	10	8.2	1.4	6.8	0.7	27	27	8.3	1.2	6.8	1.1	27
$4\frac{1}{2}$	16	8.4	1.1	7.0	1.1	12	12	8.6	0.8	7.1	0.6	28	28	8.5	1.0	7.0	0.9	28
5	19	8.7	1.5	7.2	1.2	15	15	8.5	1.0	6.8	0.8	34	34	8.6	1.3	7.2	1.0	34
6	20	8.9	1.5	7.1	1.6	17	17	8.8	1.1	6.9	0.9	37	37	8.8	1.3	7.0	1.3	37
7	22	9.0	1.6	7.2	1.2	16	16	9.0	1.2	7.1	0.8	38	38	9.0	1.4	7.2	1.0	38
8	21	9.2	1.6	7.3	1.4	16	16	9.1	1.3	7.2	1.1	37	37	9.2	1.4	7.3	1.3	37
9	21	9.5	1.6	7.4	1.4	17	17	9.2	1.3	7.2	0.9	38	38	9.3	1.5	7.3	1.2	38
10	22	9.6	1.5	7.4	1.3	18	18	9.3	1.4	7.3	0.9	40	40	9.5	1.5	7.3	1.7	40
11	21	9.5	1.5	7.5	1.6	18	18	9.6	1.2	7.3	1.0	39	39	9.5	1.3	7.4	1.4	39
12	19	9.8	1.4	7.4	1.3	18	18	9.7	1.3	7.5	1.0	37	37	9.8	1.3	7.5	1.2	37
13	20	10.0	1.4	7.6	1.4	17	17	10.1	1.4	7.8	1.1	37	37	10.0	1.4	7.7	1.3	37
14	18	10.0	1.5	7.6	1.2	18	18	10.3	1.1	8.0	1.2	36	36	10.1	1.3	7.8	1.2	36
15	17	10.6	1.5	7.8	1.3	15	15	10.6	1.1	8.0	1.2	32	32	10.3	1.3	7.9	1.2	32
16	15	10.6	1.6	7.8	1.3	15	15	10.6	1.2	8.1	1.1	30	30	10.6	1.4	8.0	1.2	30
17	12	10.6	1.7	8.2	1.3	11	11	11.0	1.2	8.2	1.2	23	23	10.8	1.5	8.2	1.2	23
18	5	10.9	1.0	8.2	1.4	6	6	11.8	0.8	8.1	0.5	11	11	11.4	0.9	8.2	1.0	11
19	3	11.0	1.5	8.6	1.9	7	7	10.7	1.5	8.2	0.1	10	10	10.8	1.5	8.3	1.1	10

Table 2. Mean annual increments in fossa size, sexes separately and together (millimetres)

Year of life	Boys			Girls			Sexes together		
	Length		Depth	Length		Depth	Length		Depth
	No.	Mean		No.	Mean		No.	Mean	
1st	8	0.64	S.D. 0.30	7	0.60	S.D. 0.56	15	0.62	S.D. 0.44
2nd	10	0.20	0.35	7	0.51	0.41	17	0.33	0.39
3rd	11	0.14	0.39	9	0.00	0.56	20	0.08	0.47
4th	14	0.26	0.54	9	0.48	0.36	23	0.35	0.48
5th	17	0.27	0.65	15	0.27	0.39	32	0.27	0.56
6th	20	0.18	0.53	16	—	0.50	36	0.10	0.52
7th	21	0.10	0.63	16	0.01	0.37	37	0.15	0.53
8th	20	0.39	0.57	16	0.21	0.48	36	0.30	0.54
9th	20	0.14	0.56	16	0.19	0.57	37	0.17	0.57
10th	21	—	0.06	17	0.19	0.42	38	0.07	0.55
11th	19	0.15	0.55	18	0.22	0.55	37	0.18	0.49
12th	19	0.20	0.51	18	0.22	0.42	36	0.28	0.48
13th	17	0.15	0.42	17	0.35	0.45	34	0.17	0.57
14th	15	0.21	0.56	17	0.19	0.69	29	0.16	0.45
15th	14	—	0.03	14	0.10	0.32	28	0.09	0.56
16th	9	0.19	0.45	14	0.21	0.40	19	0.21	0.37
17th	4	0.00	0.71	10	0.23	0.32	9	0.07	0.49
18th				5	0.12	0.30			
						0.00			0.59
						0.09			0.30
						0.21			0.31
						0.03			0.46
						0.17			0.39
						0.31			0.45
						0.41			0.60
						0.39			0.49
						0.13			0.55
						0.12			0.48
						0.09			0.53
						0.09			0.55
						0.22			0.51
						0.41			0.45
						0.28			0.36
						0.03			0.49
						0.26			0.33
						0.76			0.64
						0.63			0.83
						0.53			0.62
						0.46			0.44
						0.37			0.39
						0.31			0.28
						0.22			0.09
						0.17			0.27
						0.12			0.11
						0.09			0.17
						0.09			0.05
						0.07			0.08
						0.02			0.10
						—			0.04
						0.31			0.19
						0.17			0.23
						0.03			0.45
						0.21			0.11
						0.36			0.14
						0.40			0.41
						0.31			0.46
						0.21			0.46
						0.09			0.09
						0.00			0.04

tracings from the first and last X-ray in the series of four different children, together with tracings of intermediate films taken at approximately 5-year intervals; the measurements made off the original films are demonstrated graphically in Fig. 7. These cases were selected because they demonstrate the individual variation in growth pattern. For instance, in case 1, increase in length continued steadily

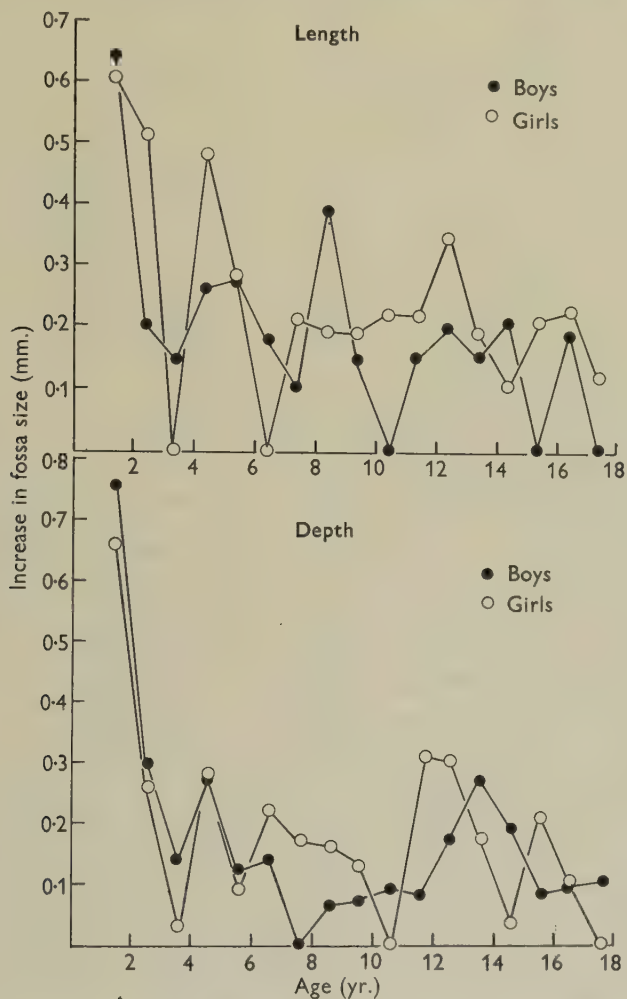


Fig. 3. Actual mean annual increments in length (above) and depth (below) of the pituitary fossa, sexes separately (see also Table 2).

throughout the entire 17 years the boy was attending at the study centre, he was also continuing to become taller and grew about an inch in stature during his seventeenth year. On the other hand, with the possible exception of his thirteenth year, when his growth spurt in stature occurred, the depth of this boy's fossa changed very little after he was aged about 8 years. In case 2, that of another boy, however, all growth in the pituitary fossa seems to have been complete by the age

Table 3. *Mean annual increase in length and depth of pituitary fossa during three phases of growth, sexes separately and combined (millimetres)**

	Boys			Girls			Sexes together		
	Length		No.	Depth		No.	Length		Depth
	Mean	S.D.		Mean	S.D.		Mean	S.D.	
Phase 1 (6th year to phase 2)	0.12	0.13	17	0.05	0.12	16	0.16	0.10	0.04
Phase 2 (year of pre-adolescent growth spurt in stature)	0.22	0.43	17	0.28	0.36	16	0.32	0.70	0.22
Phase 3 (remainder of study)	0.18	0.24	17	0.06	0.14	16	0.15	0.19	0.16
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
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							0.27	0.57	0.25
							0.17	0.22	0.11
							0.27	0.57	0.25
							0.14	0.12	0.11
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of 11 years, and there is no suggestion of the fossa undergoing a pre-adolescent growth spurt in length or depth. Growth in length is also complete early in case 3, a girl, but depth underwent a very pronounced pre-adolescent growth spurt. Case 4 is the converse to case 1, inasmuch as the greater part of growth in length was complete early in life, but increase in depth continued after puberty.

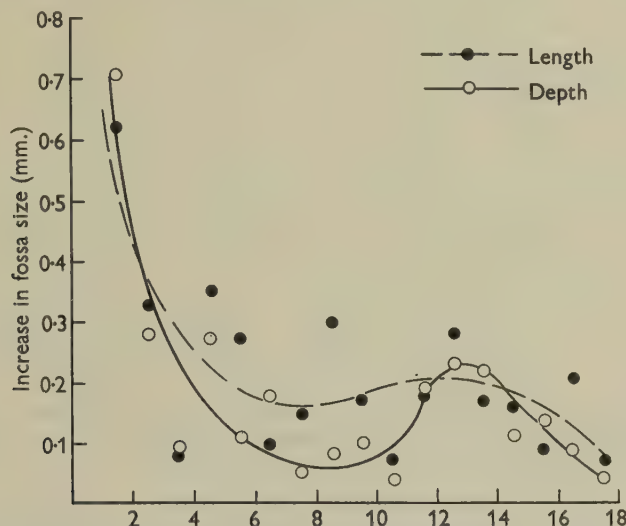


Fig 4. Actual mean annual increments in length and depth of the pituitary fossa, sexes together (see also Table 2). The curves have been fitted by eye.

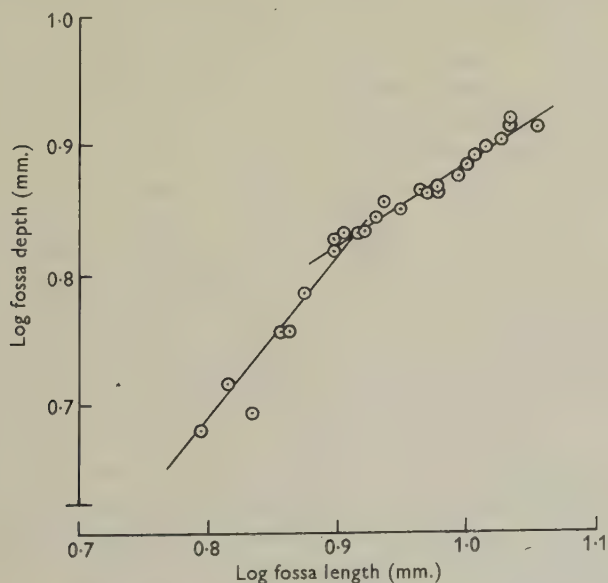


Fig. 5. Log-log plot of mean fossa depth against mean fossa length, sexes combined. Note that the slope of the line joining the first six points (i.e. for the first two years of life) differs from the slope of that joining subsequent points.

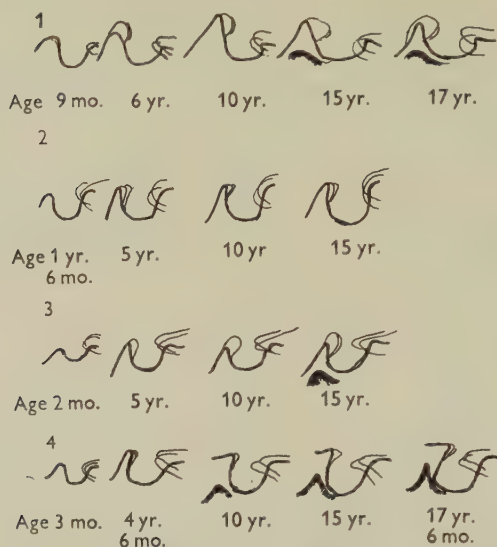


Fig. 6. Tracings of the pituitary fossae at approximately five-year intervals of four children, selected to demonstrate different growth patterns. Cases 1 and 2 are male, and cases 3 and 4 female.

DISCUSSION

The Broadbent-Bolton cephalometer permits very accurate standardization of the position of the child and of the tube film distance, and so does away with the need for elaborate geometrical procedures such as have been advocated by Lorenz (1949) and Büchner (1952, 1953), yet an error remains which is of sufficient magnitude to require consideration when increments are being studied (see Tables 2 and 3 and Figs. 3 and 4). Variations in locating the dorsum sellae and tuberculum sellae on the X-ray film are presumably the basis of the error, and may be the consequence of one of two things. First, there is always a slight blurring of the image of the bony cortex, and secondly, the morphology of some sellae is such that these two critical points may be extremely difficult to identify on the X-ray film (Burrows *et al.* 1943; Acheson, 1956; Silverman, 1957). Blurring can be, to some extent, reduced by further refinements of radiological technique such as 'orthoroentgenography' (Schaltenbrand, 1953; Nürnberger, 1955; Bergerhoff, 1956), but apart from the limited improvement in measuring technique brought by experience and practice, there is no obvious remedy for the latter difficulty.

Our finding that the standard deviations of the mean length and depth of the pituitary fossa are greater in boys than in girls is consistent with the fact that for height (Acheson, Kemp & Parfit, 1955; Hammond, 1957), skull circumference (Westropp & Barber, 1956; Falkner, 1958) and several other body measurements made in longitudinal growth studies (Hammond, 1955; Tanner, Healy, Lockhart, Mackenzie & Whitehouse, 1956; Falkner, 1958) the range, with age held constant, in boys tends to be greater than in girls.

Setting aside embryonic development, which has been studied by Covell (1927), the present analysis shows that the pituitary fossa, in common with the rest of the

skull, and most of the other skeletal components of the body, undergoes its greatest increase in size during the first 5 years of life. During these early years growth in depth is proceeding more rapidly than growth in length and statistical tests suggest that, as a rule, there tends to be a subsequent increase in the rate of growth in depth at the time of the pre-adolescent growth spurt in stature. However, although

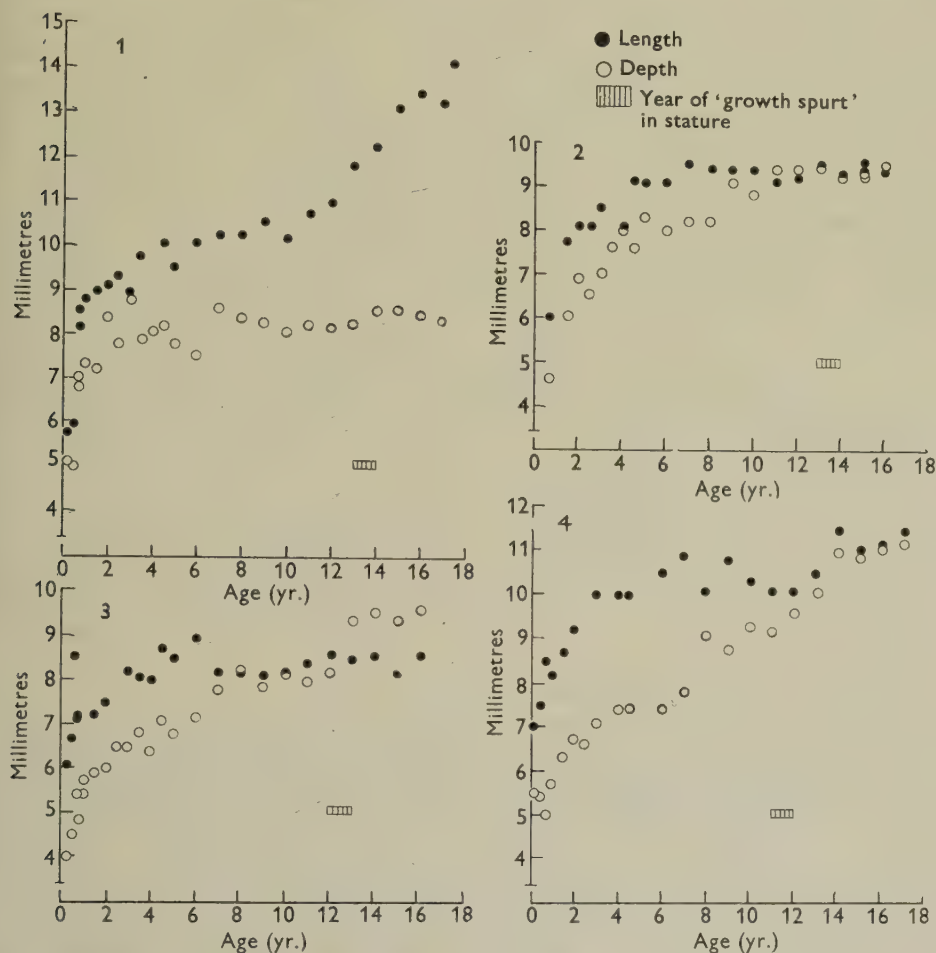


Fig. 7. Graphic record of the length and depth measurements made from the original radiograms of the cases whose fossae are shown in Fig. 6.

the latter statement is true of the group as a whole, it does not apply in every case, and in some children (e.g. Figs. 6 and 7, case 1) there was an increase in the rate of growth in length just before puberty, and in case 2 all fossa growth had ceased by the age of 11 years.

A question of some interest is how the increase in size of the pituitary fossa occurs. In some cases, for instance case 1, Fig. 6, growth in length could only have been accomplished by osteoclastic activity, involving the resorption and remodelling of

either the anterior or posterior wall of the fossa. There are two reasons which suggest that major changes do not take place in the anterior part of the fossa; first, although the anterior clinoids are remote from the fossa, their shadows on the successive X-ray films remain remarkably constant in relation to the shadow of the anterior wall of the pituitary fossa. If the anterior wall of the fossa was being eroded one would expect the anterior clinoids to appear to overlap the fossa to an ever greater extent as the child becomes older. Secondly, any alteration in the situation of the anterior wall of the pituitary fossa relative to the rest of the sphenoid bone would presumably alter the relation of the fossa to the optic chiasm, which lies on top of the tuberculum sellae, so that the chiasm would tend to become 'undermined'. This does not, in fact, occur. These suggestions find support in the work of Busch (1951), who concluded that the anterior wall of the fossa changes little in its relations throughout the ageing process. On the other hand, there is no structure lying in relation to the dorsum sellae which is likely to suffer if the dorsum moves back 0.5–1.0 cm. during the growing years, for the brain-stem will already be adapting its alignment to growth, which on recent evidence from quadrupeds (Baer, 1954; Mednick & Washburn, 1956), is occurring at the occipito-sphenoidal suture. Study of a small collection of dried skulls of children of various ages also favours the suggestion that the dorsum sellae 'moves back' during the growing period (Acheson, unpublished data).

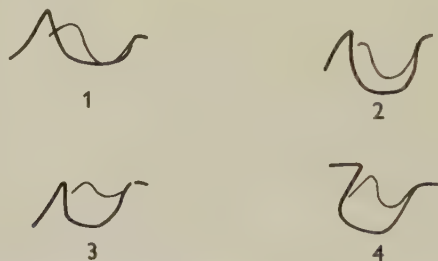


Fig. 8. The first and last tracings of the four series shown in Fig. 6. The drawings are made on the assumption that the tuberculum sellae is a 'fixed point', and they show how the growth of the fossa entails hollowing out of the floor, and remodelling of the dorsum sellae.

Thus in Fig. 8, superimposed tracings of the first and last X-rays of each of the cases discussed above are shown, and the drawings are made on the assumptions that the tuberculum sellae is a 'fixed point', and that growth is due primarily to osteoclastic activity in other parts of the fossa. Increase in depth in some cases (Fig. 8, cases 2 and 3) would seem to be due to a hollowing out of the floor of the fossa, but in others (Fig. 8, cases 1 and 4) upward growth of the dorsum sellae is also evidently an operative factor.

In conclusion, therefore, the present study supports Tanner's (1955) statement that the pituitary fossa increases its rate of growth during the pre-adolescent period, and it is shown that increasing depth contributes to this phenomenon to a greater extent than increasing length. It would, however, have been more accurate if he had written 'this structure... (is set) aside from the *vault* of the skull in this respect', for Tanner (1955, p. 13) himself believes that the mandible, the maxilla and parts

of the cranial base also grow rapidly prior to and during adolescence, a conclusion which is being borne out by the Bolton Study (Broadbent, 1956). It would seem that the growth of the pituitary fossa is largely due to osteoclastic activity with remodelling of the dorsum sellae.

SUMMARY

In the Bolton Study of dental and facial development a group of children have had their skulls radiographed at regular intervals from infancy to maturity under rigidly standard conditions. The length and depth of the pituitary fossa were measured from X-rays of 40 of these children (22 boys and 18 girls). Statistical analysis of the data shows that, as a rule, rate of growth in depth but not length undergoes a significant increase during the same year as the child experiences its pre-adolescent growth spurt in stature. Study of individual cases shows that the growth pattern varies considerably, and it is concluded that increase in size of the fossa is due to resorption and remodelling of the dorsum sellae, and osteoclastic activity in the fossa floor.

We are most grateful to Dr Holly Broadbent, Director of the Charles B. Bolton Fund, for permitting us to study the X-rays, and to Dr Normand Hoerr for giving us access to the children's stature measurements which were recorded by the Brush Foundation. Mr William Golden kindly made the tracings from which Figs. 6 and 8 were subsequently prepared, and Mr David Hewitt gave statistical advice. One of us (R. M. A.) performed the earlier part of this research during tenure of the Radcliffe Travelling Fellowship, University College, Oxford, and a Rockefeller Travelling Fellowship in Medicine; we also received a grant from the Medical Research Council of Ireland.

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THE MEDIAL INCLINATION OF THE HUMAN THORACIC INTERVERTEBRAL ARTICULAR FACETS

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INTRODUCTION

The human inferior thoracic intervertebral articular facets are very slightly concave in both the vertical and transverse planes; they face forwards with some medial and a little inferior inclination; the superior articular facets are shaped to conform with them.

Humphry (1858) stated that a pair of thoracic facets 'may be regarded as forming two portions of one circle, the centre of which is about the middle of the body of the vertebra, or an approximation to this'. Holden (1887) agreed, but Hughes (1892) thought, that if indeed the facets lie on the circumference of one circle, the centre of that circle must lie in front of the vertebral body.

It is generally agreed that the directions of movement at a given intervertebral articulation are controlled principally by the shape of the articular facets, the mode of rotation of the thoracic column being determined principally by their medial inclination. Experiments carried out by Hughes (1892), Lovett (1900, 1905, 1906), Novogrodsky (1911), and Andersson & Eckström (1941) have demonstrated that considerable rotation occurs between successive thoracic vertebrae from the second to the ninth inclusive, and that below this the amplitude decreases, there being little rotation at the thoraco-lumbar transition. Lovett also noted that rotation is accompanied by lateral flexion, an observation confirmed by Dittmar (1931) and Arkin (1950). Several explanations of this phenomenon have been put forward (Lovett, 1906; Feiss, 1907; Rogers, 1933; and others), the theories, which are not fully accepted, being based on the behaviour of series of blocks of wood or of rods of pliable material. Little attention appears to have been paid to the medial inclination of the articular facets, a feature which must be considered in any attempt to resolve the problem of factors responsible for the association of rotation and lateral flexion in the thoracic spine. To this end the inclination of the thoracic intervertebral facets has been measured in seventeen vertebral columns.

MATERIAL

Seventeen macerated and dried adult vertebral columns were selected, all columns being serially complete, undamaged, and free from congenital or pathological abnormality. Parts of two freshly macerated young adult male columns were also used in the investigation.

THEORETICAL CONSIDERATIONS

The object of the experiments was to determine the degree of medial inclination of the facets in relation to the position of the vertebral bodies, in such a manner that the results were comparable between different vertebrae and between different

columns. In Fig. 1, the dotted lines ab and cd are the chords of the arcs formed by the articular surfaces of a pair of facets of a thoracic vertebra. The extensions of these chords meet at g , forming the *facetal angle*, agd . The facetal angle gives the absolute extent of medial inclination, but does not indicate the relationship of this to the vertebral body. If, however, one considers the bisectors of the two chords, EX and FX in Fig. 1, it is apparent that the medial inclination of the facets causes the bisectors to meet at the point X , which will be referred to as the *intersection point*.

If the two facets do lie on the circumference of one circle, then X is the centre of that circle. If the facets form arcs of two different circles, as in Fig. 2, then the intersection point X will bear no clear geometrical relationship to either of the two circles p and q , but will still indicate the point towards which the facets face (Fig. 2).

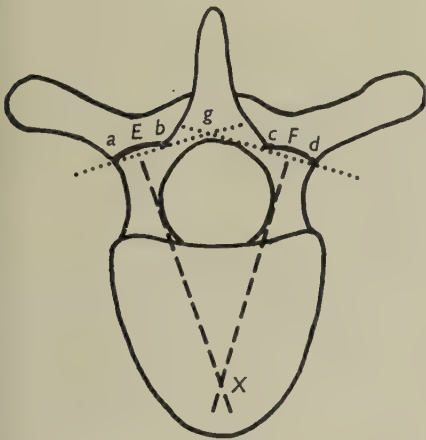


Fig. 1



Fig. 2

Fig. 1. Diagram of the lower aspect of a thoracic vertebra, showing the construction lines used in finding the intersection point, X . The curvature of the articular facets has been exaggerated.

Fig. 2. Diagram showing that an intersection point, X , can be obtained even though the articular facets, ab , cd , lie on two separate circles, p , q .

By measuring the midline distance from the vertebral lamina to the intersection point, and to the posterior margin of the vertebral body, and expressing these as a percentage of the midline distance between the lamina and the anterior border of the vertebral body, the position of the intersection points can be expressed in relation to the position of the vertebral body, and intersection points in different vertebrae can thus be compared.

METHOD

The *inferior* intersection point (X in Fig. 3) was determined by finding the medial inclination of the inferior articular facets, using engraved Perspex sheets as shown.

The *superior* intersection point was determined similarly; parallel-sided strips of Perspex, adherent to the undersurface of the Perspex sheets, were helpful in gauging the medial inclination of the facets (see Fig. 4).

The position of the mid-points of both superior and inferior facets and the chords of the superior facets had to be judged by eye, but it was found in a series of preliminary tests that the results were reproducible for any one vertebra, the method having an apparent error of $\pm 2\%$.

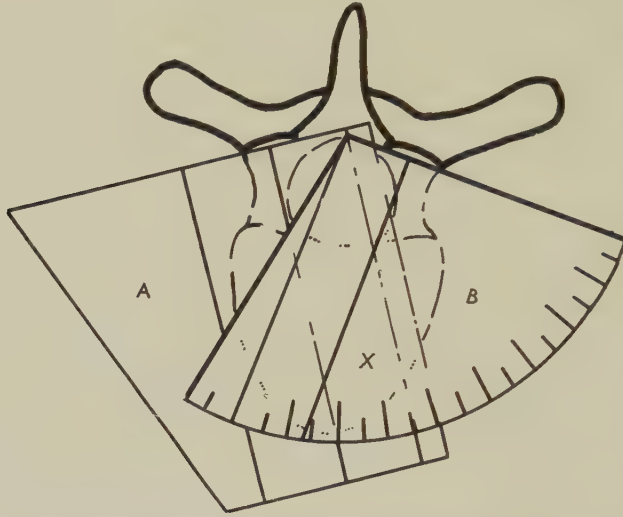


Fig. 3. Diagram to show the use of the Perspex instruments *A* and *B* to obtain the inferior intersection point, *X*, of a thoracic vertebra. The curvature of the articular facets has been exaggerated.

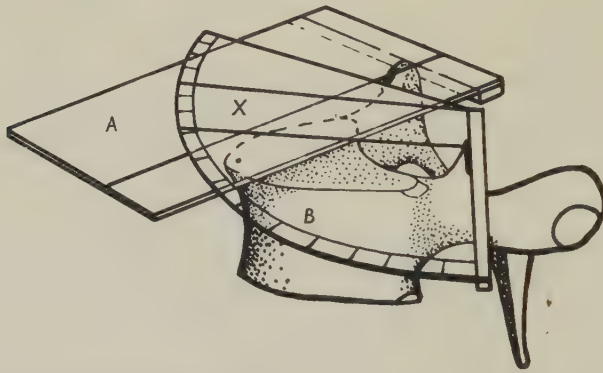


Fig. 4. Diagram to show how the Perspex strips on the undersurface of the instruments *A* and *B* were placed against the surfaces of the superior articular processes. The plane in which the instruments are lying is parallel to that of the upper surface of the vertebral body, and the point of intersection of the two lines at *X* is immediately above the intersection point on the vertebral body.

In order to make direct comparisons of the data from each vertebra, three indices were then calculated in each case, these being the upper and lower *intersection indices* and the *laminar-body length index*.

The intersection indices for the upper and lower surfaces are expressed thus:

$$\frac{\text{midline distance from lamina to intersection point} \times 100}{\text{midline distance from lamina to anterior border of body}}$$

(see $(LX \times 100)/LA$ in Fig. 5). The laminar-body length index is expressed as:

$$\frac{\text{midline distance from lamina to posterior border of body} \times 100}{\text{midline distance from lamina to anterior border of body}}$$

(see $(LP \times 100)/LA$ in Fig. 5). The measurements and the indices were tabulated. By plotting the results, a series of graphs was obtained which gave the relationship between the intersection point and the vertebral body for each vertebra measured, the results being comparable between different vertebrae and different columns.

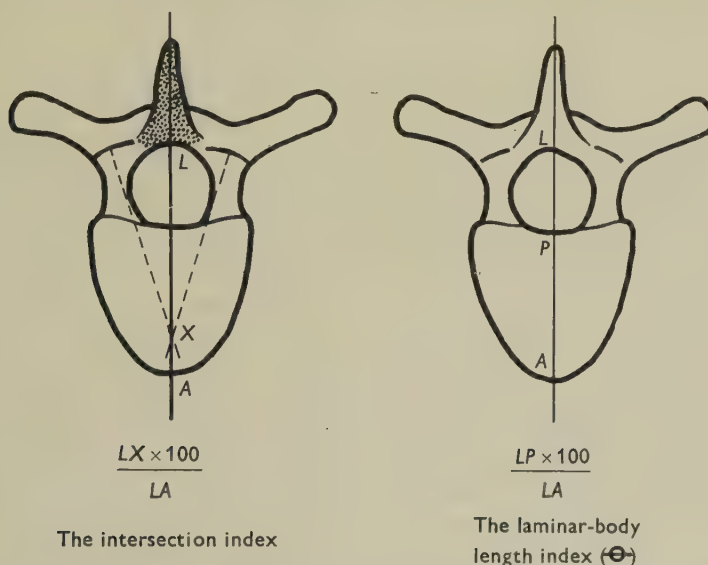


Fig. 5. Diagram to show the dimensions used to obtain the intersection and laminar-body length indices.

RESULTS

In the macerated and dried columns the facet angles of the first thoracic vertebrae were so large in three that the intersection point could not be determined. The shape and inclination of the facets of the thoraco-lumbar mortice articulation precluded the determination of the intersection point in a further three columns, and no observations could be made on the joints below the transitional level. In the seventeen dry columns, 166 joints were thus available for measurement. In one wet specimen measurement was possible at eight of the ten typical thoracic articulations, while in the other, for technical reasons, measurement was only possible at alternate joints between the second and tenth thoracic vertebra.

At all but three of these 178 articulations the intersection point for the superior facets of the inferior vertebra forming the joint lay nearer the lamina than did the intersection point for the inferior facets of the upper vertebra. The three exceptions

occurred at one articulation in each of three dry columns, at T2-3 in one, T8-9 in the second and T9-10 in the third. At no joint did the intersection points lie behind the junction of the anterior two-thirds of the vertebral body with the posterior third.

Comparison of the intersection points at different levels showed that in all columns they lay in front of the vertebral bodies in the upper one or two thoracic vertebrae. Below this, ten dry and the relatively complete wet column (Fig. 6a)

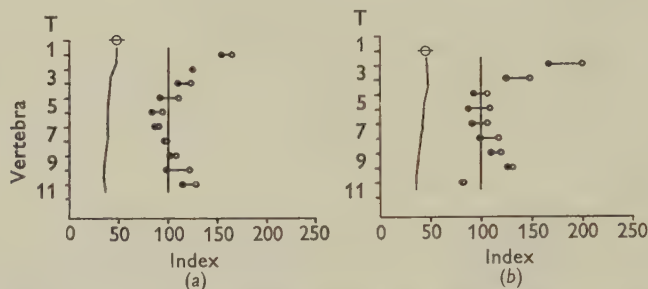


Fig. 6. The relative positions of the superior intersection index (●) and the inferior intersection index (○) at each of the thoracic intervertebral joints in two vertebral columns. Index 0 indicates the position of the anterior surfaces of the laminae in the midline. ⊕ indicates the posterior margins, and index 100 the anterior margins, of the vertebral bodies.

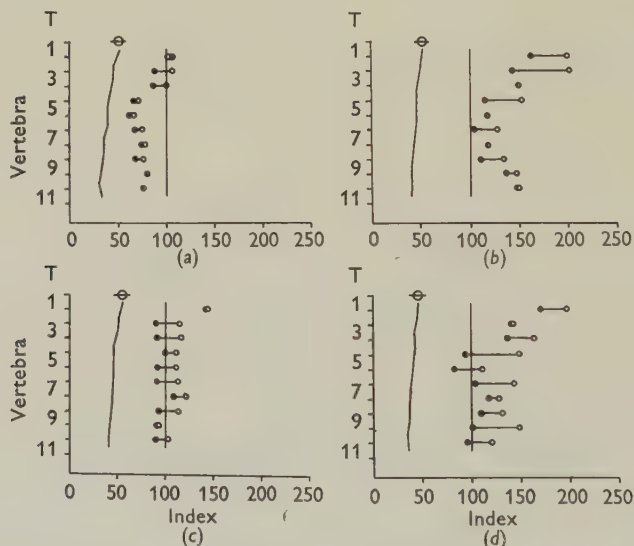


Fig. 7. The positions of the intersection indices in four columns. The symbols have the same meaning as in Fig. 6.

had a similar pattern of change, in that the intersection points approached the anterior border of the column of vertebral bodies, and either the superior or both intersection points entered the lines of the bodies; lower still the points become more anterior and left the line of the bodies again. In four of the ten dry columns (Fig. 6b) the intersection points for the joint between the tenth and eleventh thoracic

vertebrae lay more posteriorly than those for the joint immediately above, re-entering the line of the bodies.

Three dry columns (Fig. 7*a*) had a pattern similar to that of the preceding ten, in that the points entered the line of the bodies in the upper part, but differed below since the points remained within the line of the bodies. The available articulations in the second wet specimen were of similar inclinations.

Of the four remaining columns, one (Fig. 7*b*) had points above and below which were farther forward than the mid-thoracic points, but at no level did they enter the bodies; two (Fig. 7*c*) had points which lay close to the anterior line from the second thoracic joint downwards; and one (Fig. 7*d*) had points which entered the column below the fourth thoracic vertebrae, left it below this level, and re-entered the column below the ninth thoracic vertebra.

DISCUSSION

The effects upon bony dimensions of maceration and drying have been studied by Ingalls (1927) and Todd & Pyle (1928), who found that the amount of shrinkage produced was relatively small. The articular cartilage of the intervertebral facets is thin and appears to be evenly spread over the bony surface. Drying and cartilage deprivation thus appear unlikely to have had any material effect on the results obtained in the dry columns.

It has been shown that the majority of intervertebral joints in the thoracic region have a greater intersection index for the inferior facets of the upper participating vertebra than for the superior articular facets of the lower participating vertebra. So that if the pairs of facets do lie on single arcs, then the radius of the arc of the pair of inferior facets of the upper vertebra is greater than the radius of the pair of facets with which they articulate: this means that there is a certain looseness of fit, a looseness described by MacConaill (1953) as incongruence, and as a characteristic of synovial joints. Clearly this incongruence will permit lateral flexion during rotation, but by itself cannot cause this associated movement.

If it is accepted that the articular facets constitute a guiding element during vertebral rotation, one may deduce that the centres of rotation of the upper thoracic vertebrae are well in front of the vertebral bodies in most cases: in the mid-thoracic segments the centres approach and in many cases lie within the line of the bodies: and in the lower thoracic segments in many columns the centres again lie in front of the vertebral bodies, with the exception of some of the lowest typical thoracic joints. Owing to the thoracic kyphos, the upper and lower thoracic vertebrae lie in a plane anterior to that of the middle members of the series; the anterior position of the axes of rotation in these upper and lower thoracic segments must therefore be even more marked when related to the intact body. Superiorly, and in some cases inferiorly, where the axis of rotation lies well in front of the bodies, simple rotation should be accompanied by a sideways shearing movement of the vertebral bodies, whereas in the middle of the region the centre of rotation must lie in the intervertebral discs. The thinness of the thoracic intervertebral discs must prevent any great lateral shearing at the upper and lower joints.

It is clear that the two original opinions of Humphry and Holden, and of Hughes,

are not as opposed as they at first sight might have seemed. Humphry and Holden, who thought the arcs were centred within the vertebrae, were correct in so far as the middle thoracic vertebrae are concerned, and Hughes, who placed the centres in front of the bodies, was correct as far as the upper and some of the lower thoracic vertebrae are concerned.

The variability in behaviour of the intersection points in this series may well be due to sexual or racial differences, but there is no evidence available as to the effect of these factors.

SUMMARY AND CONCLUSIONS

The medial inclination of the thoracic intervertebral articular facets is best measured by determining the position of the intersection points.

In seventeen complete dry and two incomplete wet columns this measurement has revealed a considerable degree of individual variation. Commonly the upper one or two thoracic articulations have points well in front of the vertebral body, those below approaching and then entering the line of the vertebral bodies; in the lower part of the region the points again leave this line in most cases, except that the lowest thoracic joint may have points which re-enter the line. Simple rotation can thus occur in the mid-thoracic region, but elsewhere rotation must be accompanied by a lateral shearing of the bodies. The incongruity of the pairs of articulating facets is sufficient to permit associated lateral flexion of the column, although it is not necessarily a cause of this movement.

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THE EFFECT OF LOAD CARRIAGE ON NORMAL STANDING IN MAN

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INTRODUCTION

It is a commonplace observation that carrying a heavy load on the back causes the carrier to lean forwards, and that in general, the heavier the load, the farther forward does the carrier lean. The addition of a load to the back causes the normal erect posture to be so modified that the vertical projection at the feet of the centre of gravity of the man plus the load is made to approximate in position to the centre of gravity of the unloaded man (Hellebrandt, Fries, Larsen & Kelso, 1944). Indeed, it has been suggested that the location of the centre of gravity is a 'physiological constant' which it is impossible to disturb significantly in a standing man (Hellebrandt, 1950).

The manner in which the body alters its normal alignment to achieve stability under conditions of load carriage does not appear to have been examined in any detail. The present study is concerned with measuring the effect of load carriage and load position on the normal standing posture of young adult males, using a miniature camera photographic technique. The experiment was designed to find out in what way varying loads affect normal standing, and whether the same load has comparable effects on different subjects. The study was carried out on a relatively small group of subjects. The results were sufficiently clear-cut, however, to suggest that the findings have general application for conditions of static loading.

METHODS

The experiments were performed on ten male medical students, aged 18–21 years. Their heights ranged from 170.6 to 188.9 cm. and their weights from 59.75 to 84.75 kg. Four bony landmarks, which were readily identifiable, were found and marked on all subjects. The four landmarks used were: (1) the lateral side of the greater tuberosity of the right humerus ('shoulder'); (2) the lateral side of the greater trochanter of the femur ('hip'); (3) the knee joint immediately above the head of the right fibula ('knee'); and (4) the lateral malleolus of the right fibula ('ankle'). Small black adhesive markers were placed as accurately as possible on the landmarks. The subjects were then photographed from their right side against a white screen (Pl. 1).

The subjects stood on a rigidly constructed platform, with the contour of their heels placed posteriorly along a line marked on the platform. The heels of the subjects were 20 cm. apart, but they were allowed to spread their feet at will. The subjects were asked to adopt a comfortable standing posture and not to move the

position of their feet. They were placed facing at right angles to the camera, and behind them in line with their right shoulder hung a vertical plumbline. Alongside the plumbline was placed a metre calibration rule, which appeared in every picture taken (Pl. 1). The camera was placed $3\frac{3}{4}$ m. from the subject, and exposures were made using an electronic flash, with a flash duration of approximately $\frac{1}{2000}$ th of a second. The camera used was a Leica III f , with a Summicron 5 cm. lens.

Each subject was photographed under the following conditions: (1) normal standing, without load; (2) carrying an empty carrier; (3) carrying 12 kg. attached to the carrier high on the back; (4) carrying 24 kg. as in (3); and (5) carrying 24 kg. attached to the carrier low on the back. The load of 24 kg. was moved over a distance of 30 cm. between the 'high' and 'low' positions (Pl. 1, figs. 3, 4). The carrier used for the experiments was adapted from a standard Bergen rucksack frame. Adjustable shoulder straps and waist belt were fitted, and on the back of the frame two vertical steel bars were welded, over which a platform carrying the weights could be moved. The weight of the carrier itself was 4.25 kg. and additional weights of 2, 4 and 6 kg. were used. The weight of the load was concentrated into a relatively small mass, and the approximate centre of gravity of the load in relation to the anatomical markers, is shown diagrammatically in Text-fig. 2. The shoulder straps of the carrier were adjusted individually for each subject, so that the waist-band supporting the load at the back fitted the natural waist of the subject.

For the experiments the subjects wore only trunks and vest. Subjects were photographed twice, but no subject came twice on the same day. It is considered that the heel positions on the platform did not vary over more than 0.5 cm. from subject to subject. Subjects were photographed about half a minute after they had been placed in position, for each of the successive load positions. The exact timing of the photographs varied slightly, and the release mechanism was operated surreptitiously. This, combined with the fact that he was not facing the camera, reduced the possibility of posing on the part of the subject.

The same procedure was then adopted with the subject carrying first 12 kg. and then 24 kg., without moving the position of his feet. The carrier was loaded from behind, and the subject did not have to move in any way. The weight-carrying platform on the carrier was used in only two positions—its upper and lower limits, which were 30 cm. apart ('high' and 'low' positions). The centre of gravity of the load in the high position approximated to the level of the shoulder joint; in the low position, it approximated in position to the lower lumbar region of the subject (Pl. 1, figs. 3, 4).

The film negatives were enlarged using a Leitz microfilm reader, and the size of the enlarged image was about one-tenth of the actual size of the subject. Direct measurements were made on the enlarged image, which was projected on to a perfectly flat white screen. A ruled grid was used for measuring, one edge being lined up along the plumbline, and the other edge at right angles to the plumbline, along a line marking the position of the right foot on the platform. The vertical height above the platform, and the horizontal distance from the plumbline could thus be measured for each of the four markers. The length of the metre rule was also measured on each negative, and this figure was used as the calibration factor. The position of the markers in relation to the two reference lines on the negative

(plumbline and platform) was measured to an accuracy of half a millimetre on the enlarged image.

There are several potential sources of error in this technique, but the close similarity of the results for the two experiments (see Table 1) suggests that these errors have been largely avoided. The errors relating to the photographic distortion in measuring from 35 mm. negatives have been discussed by Tanner & Weiner (1949), who found distortions due to camera lens error, and differential distortion of the negative image during development to be immeasurably small. The chief error is likely to arise during printing of the negative, and this was avoided by measuring from the projected negative image. The error due to parallax was minimized by measuring the four markers from a plumbline in the same plane as the markers. As the metre rule appeared in all the pictures taken, all measurements were related to a calibration factor in the same sagittal plane as the markers.

Probably the main source of subject error arises from the fact that the subject never remains completely still. It is well recognized that the body sways continuously, especially in an antero-posterior direction (Hellebrandt & Franseen, 1943), and it is theoretically possible that the pictures taken of the same subject under identical load conditions on different days may have been taken at the extreme limits of the forward and backward sway, respectively. The fact that the results show precisely the same trend for the second occasion on which the subjects were photographed as they do for the first occasion, and that the actual figures are in general very similar, suggests that the differences due to the subjects having been photographically 'frozen' in motion during varying parts of the postural sway cycle are small in relation to the effect of load carriage.

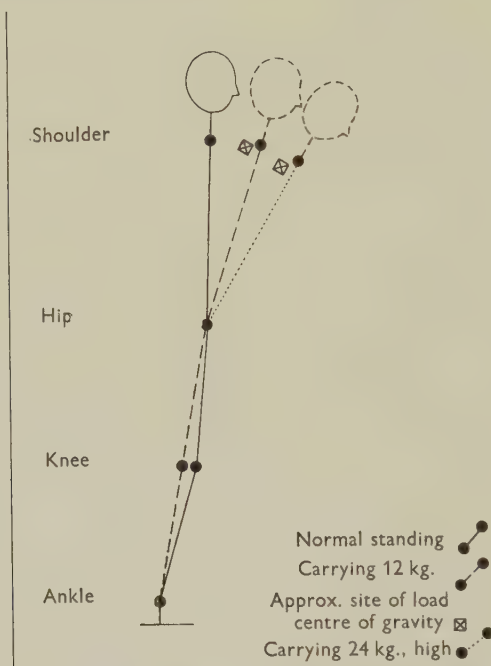
The analysis of the results was performed by comparing the positions of the four anatomical markers on each subject under varying load conditions. This was done for each subject on two occasions, so that there was a total of forty individual readings for each subject. Analysis of the data showed that there was no significant difference in the position of the ankle under the varying load conditions. The ankle marker was therefore taken as the zero position, and the positions of the other three markers were estimated in relation to a vertical line through the ankle marker. The results were compared by an analysis of variance, and the levels of significance determined by using a *t*-test.

RESULTS

The results showed a highly significant ($P < 0.001$) forward displacement of the shoulder with loading, and the same load carried in the low position caused a further displacement forward than when carried in the high position. The unloaded carrier, however, made no significant difference to the position of any of the markers. The position of the hip marker did not move significantly for the varying load conditions, but the position of the knee marker moved progressively backwards with increasing load carriage ($P < 0.001$). There is therefore a highly significant re-alignment of the body segments, as represented by the anatomical landmarks used in these experiments, with the carriage of loads above a certain minimum weight in the standing position. Text-figs. 1 and 2 illustrate diagrammatically the re-alignment that occurs.

The greatest amount of movement took place at the shoulder, as would have been

expected. There were highly significant differences in position between normal standing and carrying the carrier plus 12 kg., between carrying 12 kg. and 24 kg., and between carrying 24 kg. in the low as compared to the high position ($P < 0.001$ in all cases). Thus the heavier the load, provided at least that it exceeded the weight of the carrier (4.25 kg.), the further forward did the subject lean, and for the same weight, the lower position on the back caused the shoulder to be displaced further forwards.



Text-fig. 1. Diagram showing disposition of body linkage under different loads.

The position of the shoulder was also compared using the angular displacement from a vertical line through the hip marker, instead of the direct horizontal displacement of the shoulder with loading. This was done to see if the height of the subjects was a significant factor affecting the displacements. If the height of the subject was important in this respect, the effect would be most readily observable for the position of the shoulder. It was found, however, that the levels of significance between different effects of the varying load conditions were virtually the same as those obtained using the direct horizontal displacement.

The results for the position of the hip marker showed that the position of the marker did not significantly vary for any load condition investigated. While the shoulder is moving forward with increasing load, the position of the hip remains relatively fixed. There is no statistically significant difference even between the position of the hip marker when the subject is carrying 24 kg., and the position during normal standing without load. The position of the hip marker was also compared using the angular displacement from a vertical line through the ankle marker,

instead of the direct horizontal displacement. As in the case of the shoulder, it was found that the angular displacement of the hip from the ankle gave results comparable to those obtained by direct measurement of the horizontal displacement. Measured by direct horizontal displacement, or by angular displacement from a vertical through the ankle, the position of the hip marker did not significantly vary with the varying load conditions.

The results for the knee marker showed clearly that there was an over-all tendency for the knee position to move backwards with progressive loading ($P < 0.001$). Here, too, there was no significant difference between the subject standing normally without the carrier, and the subject with the unloaded carrier. But highly significant differences were found between the position of the knee in the subject standing normally (without carrier) and the position of the knee when the subject was carrying 12 kg., and when the subject was carrying 24 kg. (both positions). There was no significant difference in knee position between carrying 24 kg. in either the high or low position. In Table 1, the position of the ankle marker is considered as the zero position, and the position of the other three markers is given as the distance they lie in front of the ankle in the vertical plane. The results represent the average displacement in centimetres, for the ten subjects, of the markers under the varying load conditions.

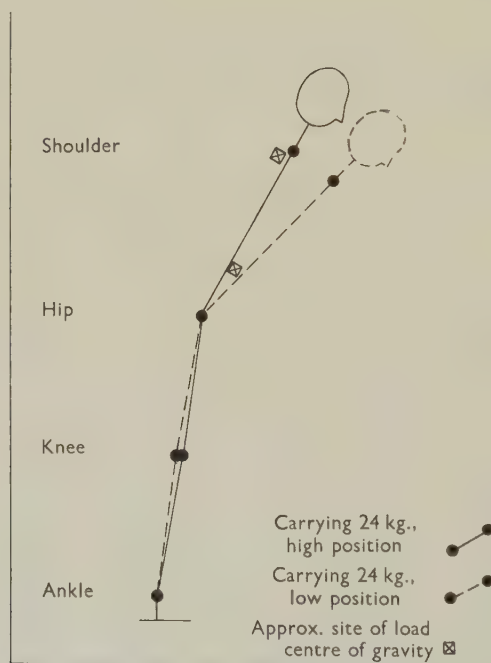
Table 1. *Average displacement (in cm.) of shoulder, hip and knee markers vertically in front of ankle marker. Data from 10 subjects in two experiments*

	Normal	Empty carrier	12 kg. (high)	24 kg. (high)	24 kg. (low)	
Exp. 1						
Shoulder	6.77	8.30	13.41	19.14	24.08	—
Hip	6.42	6.53	6.37	6.34	6.85	—
Knee	4.94	4.58	3.72	3.83	3.46	—
Ankle	0.00	0.00	0.00	0.00	0.00	—
Exp. 2						
Shoulder	7.48	8.05	13.86	18.56	24.69	—
Hip	6.51	6.42	5.48	5.83	6.26	—
Knee	4.92	4.12	2.87	2.67	2.47	—
Ankle	0.00	0.00	0.00	0.00	0.00	—
Average						
Shoulder	7.12	8.18	13.63	18.85	24.39	$P < 0.001$
Hip	6.46	6.48	5.92	5.83	6.51	N.S.
Knee	4.93	4.35	3.30	3.00	2.97	$P < 0.001$
Ankle	0.00	0.00	0.00	0.00	0.00	—

It was also found that the angle between the shoulder-hip line and the vertical, and the angle between the ankle-hip line and the vertical, showed no significant correlation with either the weight or the height of the subjects during load carriage. Under the conditions of these experiments, on the ten subjects examined, there was therefore no evidence to suggest that the posture of the subjects when loaded was significantly related to their height or weight.

Analysis of the data showed a significant difference between the average results for the position of the hip and knee for the two experiments. The average values for

the position of the markers with each load are consistently lower for the second occasion on which the subjects were photographed. However, with the shoulder position there is no significant over-all difference, four of the subjects showing a decrease in displacement in the second experiment, whereas six subjects showed an increase. These different effects are not significantly different, and so represent random fluctuations about the mean displacement. When individual subjects were compared, there was found to be a significant difference between the average displacements for the shoulder, hip and knee for the ten subjects, at the 0.1 % level. There was also a significant interaction between loads and subjects, implying that the effects of the load differ significantly from subject to subject. However, with the shoulder the trend is the same for each subject (displacement increasing with load), except in three cases in which the displacement with the carrier is less than normal. For the hip there is no consistent trend, but there is also no significant difference between the loads.

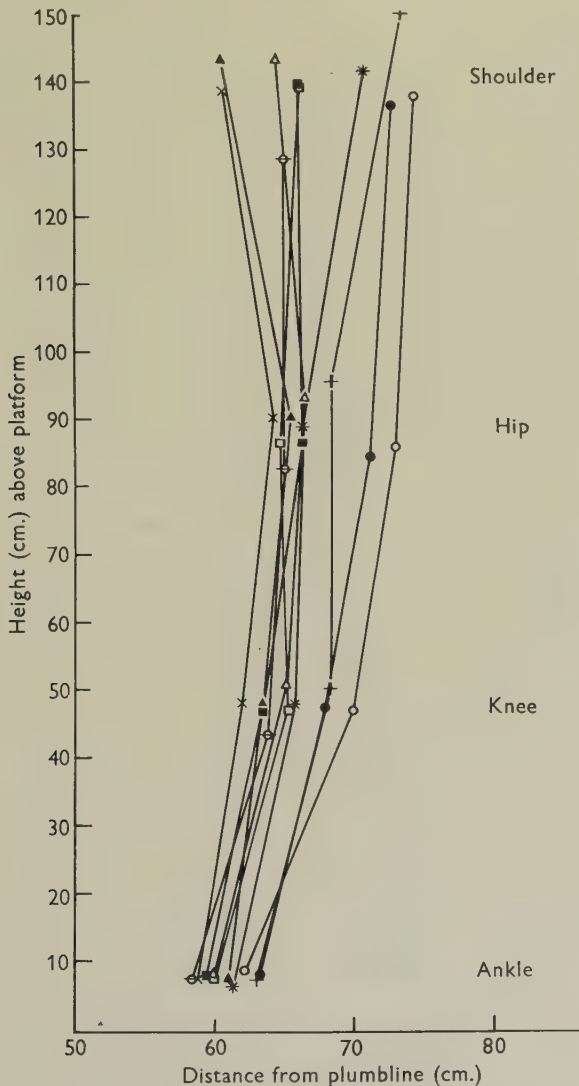


Text-fig. 2. Diagram showing disposition of body linkage when carrying 24 kg. in a high and low position on the back.

DISCUSSION

The effect of load carriage on the normal standing posture has previously been investigated chiefly by Hellebrandt *et al.* (1944) and Hale, Coleman & Karpovich (1953). Hellebrandt *et al.* claimed that the body counterbalances the effect of load carriage by leaning forwards *in toto* over the ankle joints, without significant realignment of the rest of the body. They gave few details of the technique whereby they reached these conclusions, and it is difficult to evaluate their results. Hale *et al.*

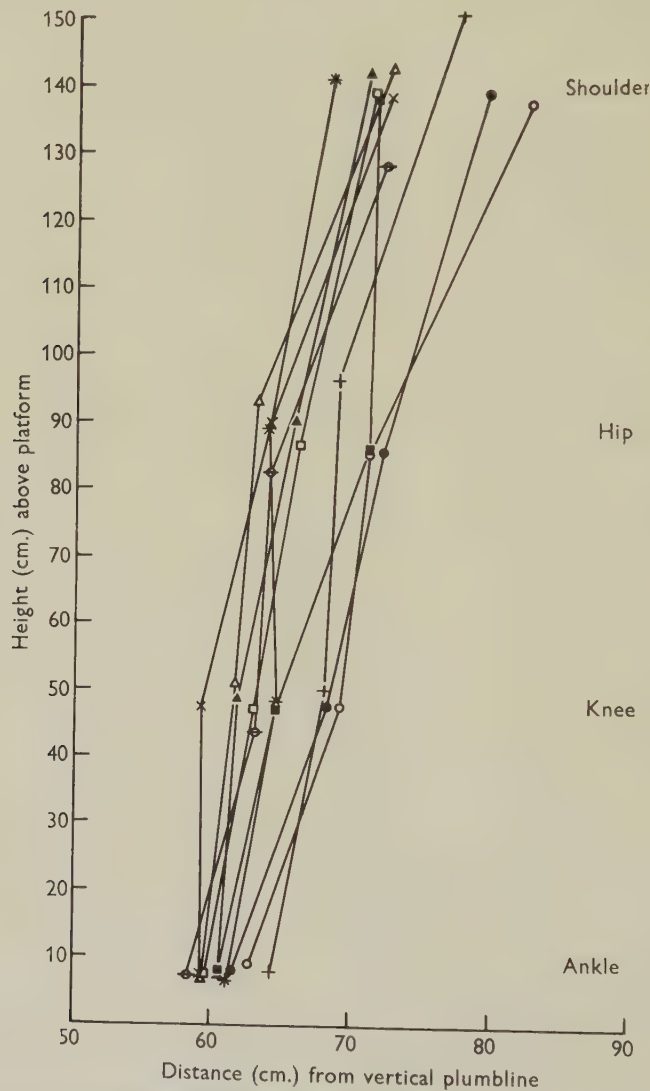
(1953) found that the carriage of a load low on the back tended to cause the body to lean further forward than a load carriage high on the back. Their results were not statistically significant, however, and they did not comment upon the effect of load carriage on the alignment of the body segments.



Text-fig. 3. Plot of the positions of four landmarks on ten subjects during normal standing.

It is apparent that the present data contradict the findings of Hellebrandt *et al.* (1944), and extend the findings of Hale *et al.* (1953). The results show that the body responds to the effect of load carriage on the back by altering the relative position of the body segments. The essential change takes place *above* the level of the hips, the trunk being displaced progressively forwards with increasing load. The plots

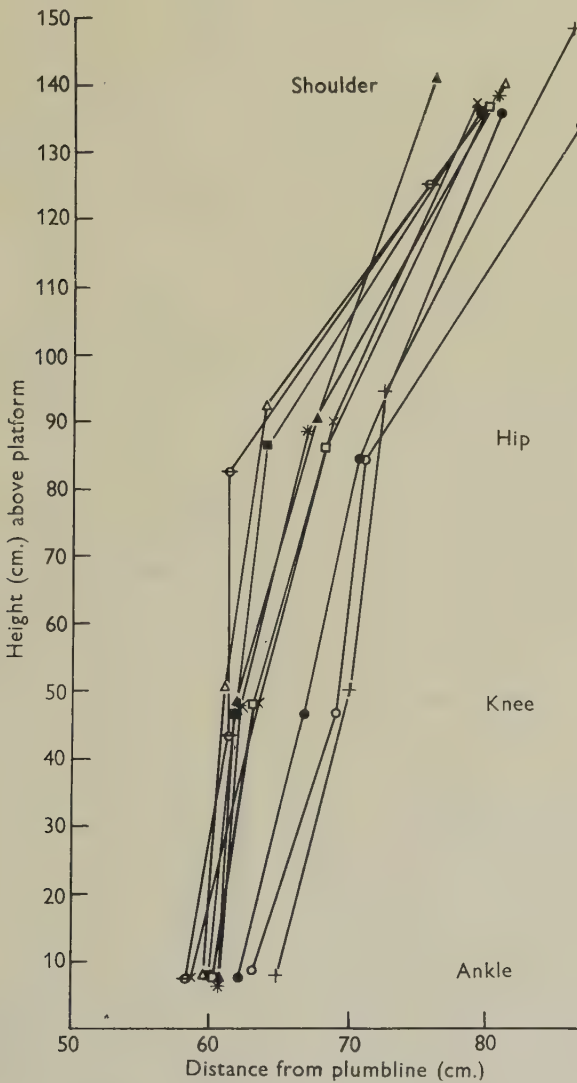
illustrated in Text-figs. 3-5 demonstrate the changes in marker positions for three of the five conditions investigated. The plots were constructed from the results of the first experiment, and were drawn accurately to scale. They demonstrate clearly that the displacement caused by load carriage occurs between the hip and shoulder



Text-fig. 4. Plot of the positions of four landmarks on ten subjects carrying 12 kg. high on the back.

markers, and that for comparable loads the general pattern is very similar for all ten subjects. One subject in Text-fig. 4 did not show any forward displacement of the shoulder with 12 kg. as compared to the normal position. However, it will be noted in Text-fig. 5 that with the 24 kg. load the shoulder marker of this subject followed the same general trend as the other nine subjects. The scatter of the points

for the positions of the various markers is due largely to variations in body stature, and to some extent to the fact that the horizontal spread of the points has been exaggerated for the sake of clarity. The results show that, for a given load, the trunk will lean further forwards if the load is low on the back than if it is in a higher



Text-fig. 5. Plot of the positions of four landmarks on ten subjects carrying 24 kg. high on the back.

position. This suggests that the body treats the load on the back as essentially a problem in balancing. If the vertical projection of the centre of gravity is kept in a more or less constant position (Hellebrandt *et al.* 1944), it follows that lowering the position of a heavy weight on the back must cause the trunk to lean further forwards to keep the combined centre of gravity of the body plus the weight in the

same position. The fact that the trunk adapts its alignment in relation to the position and weight of the load it is carrying, as has been demonstrated in the present experiments, accords with the view that the vertical projection of the centre of gravity varies within very small limits for any individual. Hale *et al.* (1953) also found that there was a general trend for a low pack to cause greater trunk inclination than a high pack, when carried on the back. Their results were not statistically significant, however.

The evidence relating to the position of the knee joint indicates that heavy loading causes a certain amount of hyper-extension of the knee joint. Whether this is a purely passive phenomenon, or whether it is at least in part due to increased muscular activity in the legs cannot be decided on the basis of the present experiments. Smith (1956) has given a detailed analysis of the passive limiting mechanism which operates at the knee joint in the normal standing position, and has emphasized that the adult knee joint is stabilized during standing by two complementary factors, the postural contraction of the flexor muscles and the passive resistance of the tissues. It is reasonable to assume that during load carriage there is increased muscular activity helping to stabilize the joint, and this increased muscular activity, together with passive stretching of the tissues, causes hyper-extension of the knee joint.

Lippold & Naylor (1950) recorded electromyographic activity in the trunk muscles under two load conditions, and reported that when a load was carried high on the back, greater activity was recorded than when the load was carried around the pelvic girdle. They suggested this result was due to the higher combined centre of gravity when a load in the high back position is carried, and this results in greater instability. To maintain the body equilibrium under these less stable conditions, increased muscle activity is required. Lippold & Naylor's results cannot be directly compared with the results of the present experiment, as they investigated somewhat different load conditions. However, as Hale *et al.* (1953) pointed out, Lippold & Naylor did not refer to the possible effects of the inclination of the trunk varying with the position of the load. It has been shown that low back carriage causes increased inclination of the trunk, and this of itself could alter the level of muscular activity. It may be, for example, that while a load carried high on the back causes increased erector spinae activity, the increased inclination of the trunk caused by carrying a load low on the back results in increased activity of the hip extensors, the over-all muscular activity remaining relatively constant. This suggestion is supported indirectly by the findings of Daniels, Vanderbie & Bommarito (1952), who found on the basis of energy expenditure experiments no significant difference in the energy cost between carrying a load in a high or low position on the back. Further indirect support for this view is gained from the work of Reid, Renbourn & Draper (1955), who observed that there were no significant differences between the physiological effects of different types of Army packs, as tested by pulse rate, rectal temperature and expired air volume. If the position and type of load carriage significantly affected the over-all skeletal muscular activity, this might be expected to be demonstrable in physiological tests.

The present experiments, in which two more-or-less extreme positions of practical load carriage on the back were adopted, suggest that as far as the anti-gravity muscles are concerned, the relative position of the load is not of primary importance.

Considering the body as a whole, a load on the back of 24 kg., for example, requires the muscles resisting the effect of gravity to work as much for one position of the load as for another. It seems reasonable to suggest that for comparable loads the *total* muscular activity necessary to resist the effects of gravity will not vary significantly, wherever the load is placed on the back. It was shown by Hellebrandt *et al.* (1944) that the combined centre of gravity of the body plus the load remains in virtually the same vertical projection as the centre of gravity of the unloaded body, and the present data show that this is accomplished by alterations in the position of the trunk. It is suggested that the trunk acts as a counterbalance, altering its inclination according to the site of the load on the back, and in this manner the projection of the centre of gravity at the feet is kept in a relatively constant position.

SUMMARY

1. The effect of load carriage on the normal standing posture has been studied using a miniature camera photographic technique.

2. The displacement from the vertical of marked anatomical landmarks was measured on enlarged negative images, during normal standing and when loads of 12 and 24 kg. were carried.

3. It was found that there was a highly significant ($P < 0.001$) forward displacement of the shoulder with load carriage, while there was no significant change in the position of the hip. There was a highly significant ($P < 0.001$) backwards displacement of the knee, while the position of the ankle was not significantly affected.

4. It was concluded that a highly significant re-alignment of the body position takes place during load carriage above a certain minimum weight.

5. A weight of 24 kg. was found to cause further forward displacement of the shoulder when it was carried low on the back than when it was carried high on the back ($P < 0.001$).

6. The suggestion is made that the trunk acts as a counterbalance, altering its inclination according to the position of the load on the back, so that the vertical projection of the centre of gravity remains relatively undisturbed.

I would like to thank Dr R. J. Whitney for having suggested this study, and for his constant help and encouragement. I would also like to thank Professor Sir Wilfrid Le Gros Clark, F.R.S., in whose Department the work was carried out. I am indebted to Miss Rosemary Morton for much statistical advice, and to Miss Christine Court for drawing the illustrations.

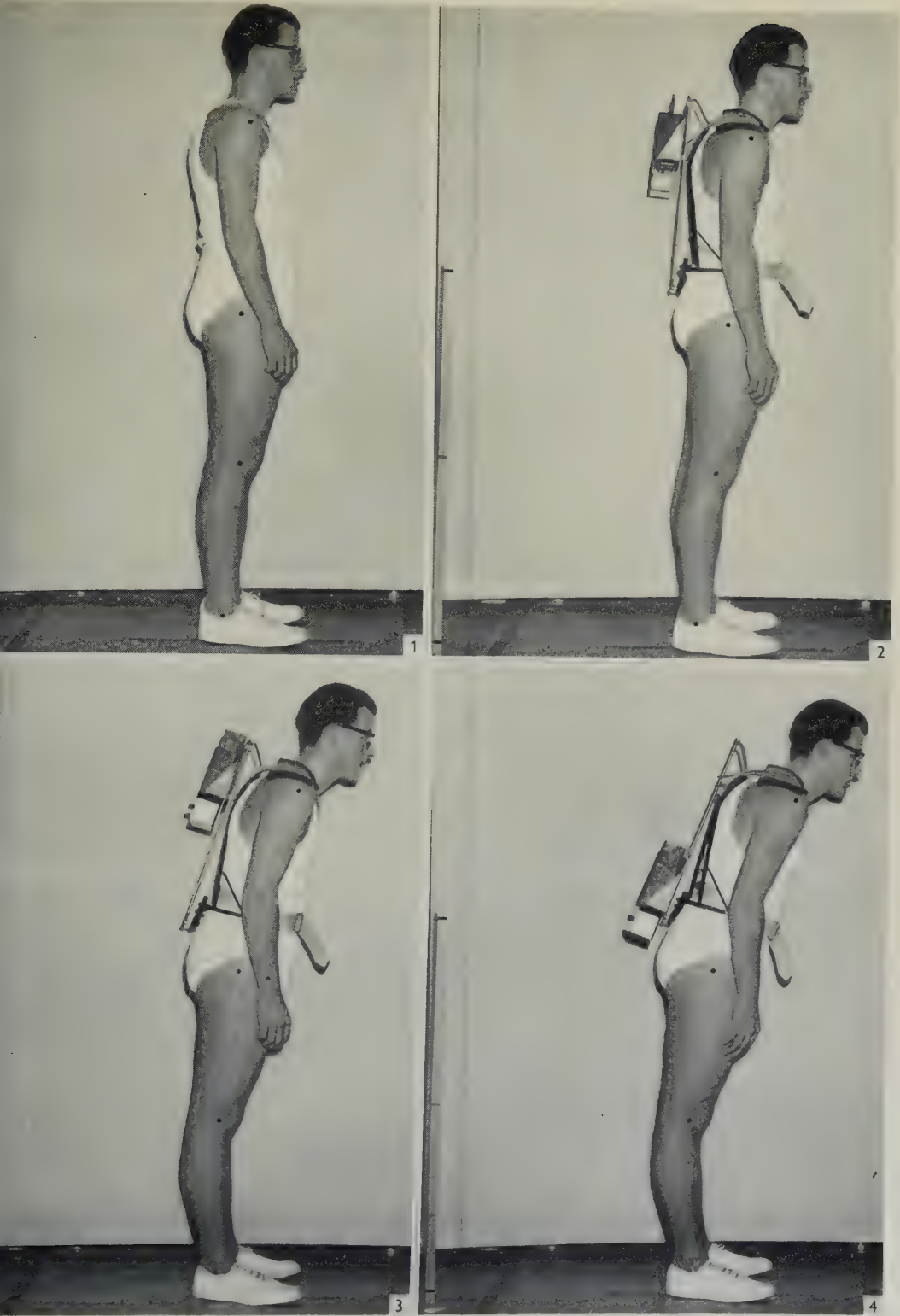
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EXPLANATION OF PLATE

- Fig. 1. Normal standing posture. The plumline and metre rule are on the left of the picture.
- Fig. 2. Carrying 12 kg. in a high position on the back.
- Fig. 3. Carrying 24 kg. in a high position on the back.
- Fig. 4. Carrying 24 kg. in a low position on the back.



DIFFERENCES IN THE TENSILE STRENGTH OF BONE OF DIFFERENT HISTOLOGICAL TYPES

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INTRODUCTION

A considerable amount of work has been done on the strength of bone (Evans & Lebow, 1951; Dempster & Liddicoat, 1952; Hülsen, 1896; for a review of the literature see Evans, 1957), and some work has been done on the strength of bone samples taken from different parts of the same bone (Olivo, Maj & Toajori, 1937). It became necessary, during the course of investigations I had been making on the structure and development of Haversian systems, to find out whether bone is strengthened or weakened by the presence of Haversian systems. It seems, however, that there is only one published work on the strength of bone of different histological types (Walmsley & Smith, 1957). In this, merely a short report of a paper read before the Anatomical Society, the authors state that they have observed differences, in Young's modulus and ultimate breaking strength, in bone of different histological types in the horse. Professor Walmsley (personal communication) writes: '...where one gets the apparently circumferential lamellae, there is a high Young's modulus and also a high breaking stress, whereas in bone of the apparently "normal" Haversian pattern, there is a low Young's modulus and a correspondingly low breaking stress.'

The present work, which shows that bone containing Haversian systems has less tensile strength than that with no Haversian systems in it, was carried out to help resolve this problem, and agrees in general with the work of Walmsley & Smith.

DEFINITION OF HAVERSIAN SYSTEMS

For the purposes of this study, the bone of the compacta of the diaphysis of the femora of oxen was considered to fall into one of two histological categories, either 'Haversian systems' or 'not Haversian systems'. The latter term groups together many types of bone structure; for instance, both the typical laminar bone of Artiodactyls and bone containing irregular primary osteones are included under this heading. However, all these types are characterized by the fact that they are primary. That is, they are not formed in the spaces left by the resorption of previously existing bone. Typical Haversian systems, on the other hand, are formed as the result of infilling, by lamellar bone, of more or less tubular cavities which have been excavated round blood vessels.

It is important to note that primary osteones are included in the category 'not Haversian systems', though in many respects they are similar to secondary osteones, which is another name for Haversian systems (Pritchard, 1956). Though these two types of structure appear rather similar under the microscope, it is in fact simple to

distinguish them from each other. Secondary osteones may be distinguished from primary osteones in the following ways.

(a) They are nearly always surrounded by a sheath of cement substance, which appears under the microscope as a bright line surrounding the outermost lamella.

(b) The orientation of the osteocytes and lamellae in the surrounding bone is not affected by the presence of Haversian systems. They appear to run straight up to the cement line and then to stop abruptly. On the other hand, the surrounding structures curve round primary osteones rather like the streamlines round the wing of an aeroplane.

(c) Few canaliculi pass from a Haversian system to the surrounding structures; they mostly do not extend beyond the cement line. There is no interruption of canalicular connexion between primary osteones and the surrounding structures.

(d) One Haversian system often encroaches on other adjacent systems, sometimes occluding the lumina of the latter. This never happens with primary osteones.

THE STRENGTH OF BONE

When an object is acted on by an external force there will be an intermolecular resistance inside the object which will tend to prevent the object deforming. The magnitude of this resistance is called the **STRESS**. The stress will, however, only tend to prevent the object deforming. The amount that the object is actually deformed is called the **STRAIN**. There are three ways in which forces can act upon objects to produce strain. They can produce compression, tension or shear. In compression the molecules of the object come closer together: in tension they separate from each other, and in shear they slide over one another. From the biological point of view it is nearly always the tension strength of bone that is important. Most accidental fractures of bone are tension fractures, even the spiral fractures associated with torsion (Evans, Pedersen & Lissner, 1951). Compressive and shearing fractures are usually the result of impact, which is relatively uncommon in nature, though in civilized human societies with their large number of high-velocity projectiles, impact fractures are more common. Even so it must not be thought that if a bone fails after an impact it has necessarily failed in compression or shear. Many of the fractures of the skull produced in motor-cycling accidents are tension failures (Evans, 1957).

The reason for tension fractures being more common than compression fractures is that bone, like most brittle substances, is stronger in compression than in tension (Evans, 1957, chap. 4). If a bone is more or less symmetrical, that is if the part subjected to great tension is not thicker or more massive than the part subject to great compression, and if it is loaded until it breaks, then naturally it will fail in tension. The human femur is nearly twice as strong in compression as it is in tension; the ultimate strength is of the order of 12,000 lb./sq.in. in tension and 20,000 lb./sq.in. in compression, and more or less the same relationship holds for oxen (Evans, 1957, chap. 14). In nature it is probable that the usual reason for the breaking of a bone is a fall, and in a fall the stresses imposed on a bone bear no necessary relation to those imposed on it during normal running and jumping.

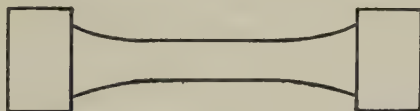
Fractures in shear must be extremely uncommon. For this kind of fracture to

occur one part of an object must be held rigidly, while the part immediately adjacent to it is subject to a force. The further the point of application of the force is moved from the rigidly held part the larger will be the bending moment and therefore the likelihood of failure in tension. As bone is nearly as strong in shear as it is in tension (Evans, 1957) the rather special conditions needed to produce a fracture in shear must be uncommon in nature.

It follows from this that the majority of failures of bone will be failures in tension, and it is therefore more informative to consider the tensile strength of bone than its strength in compression or shear.

MATERIALS AND METHODS

Femora of oxen were bought from the butcher. They had been chilled but not deep frozen. As soon as possible afterwards the test pieces were turned on a lathe. By using a fairly high rate of revolution, about 1000 revolutions a minute, it was possible to turn the bone accurately and with quite a high polish. The turning must be done carefully or the temperature of the bone will rise rapidly, thus probably affecting the properties of the collagen. The test pieces were turned in such a way that the tension stresses produced during testing acted along the long axis of the wall at the point from which the test pieces were taken. They were taken immediately above and below a point midway between the two articular surfaces. No test piece broke at a point that was in origin more than 2 in. from the middle of the bone.



Text-fig. 1. Diagram of test piece. Actual length about 1.1 in.

The shape of each piece was somewhat like a dumb-bell (Text-fig. 1). There was a lug at each end and a central straight section of uniform diameter in the middle. Between the straight section and the lugs there were gently sloping shoulders. The diameter of the central section varied from piece to piece, the smallest being 0.075 in. and the largest 0.105 in. The great majority had a diameter of from 0.095 to 0.1 in. After being turned the test pieces were put into physiological saline for 24 hr.; it is well known that dry bone is stronger than wet bone (Evans, 1957), and it is best to reproduce living conditions as far as possible.

The ultimate tensile strength of the test piece was then determined at room temperature in a Hounsfield tensometer. Care was taken to see that the test piece was kept wet during the experiment, which usually lasted about 2 min. The rate of loading was about 50 lb./min. This rate of loading was kept as high as possible, consistent with accurate reading. Some authors have pointed out that whereas dry bone behaves as an almost perfectly elastic substance, wet bone creeps. In a perfectly elastic substance strain is always proportional to stress. A substance that is imperfectly elastic will behave more or less like an elastic substance up to a certain stress, called its elastic limit, but after that point the amount of strain caused by a given increase of stress is larger. Evans & Lebow (1951) state that the

energy absorbing capacity of bone is more or less proportional to the total strain. Therefore, they say: 'The greater energy absorbing capacity of wet bone is of extreme importance in traumatic fractures, most of which arise from blows or impacts and are energy problems.' It is very doubtful, however, whether results obtained concerning the stress/strain characteristics of bone under slow loading are applicable to behaviour under impact. When a bone breaks it is usually subjected to a very large strain for less than a second, and in this time the amount of creep that can occur must be small.

When the test piece had failed it was examined, and if it had not broken in the central straight section it was discarded. The usual reason for the piece not breaking in the central portion was that it had started to fail at some little nick in the sloping shoulders that led from the lug to the central section. This part was more difficult to turn than the central section. If the test piece was satisfactory in this respect it was then sawn through as close as possible behind the break, ground in 90 % alcohol between two ground-glass plates to a thickness of about 150μ and then mounted in solid balsam.

The mounting method, for which I am indebted to Mr P. L. Small of this department, is as follows. The section is thoroughly dried. A glass slide is heated over a very low bunsen flame. Solid balsam, impaled on a seeker, is rubbed on the centre of the slide until it melts and forms a puddle about the size of a halfpenny. Bubbles will form in the melted balsam, and these must be removed by passing the slide, balsam side downwards, several times through the flame. The slide is then removed from the flame, and the section to be mounted is placed on one end of it clear of the balsam puddle. This end is then placed over the flame to heat the section. The slide is removed from the flame and allowed to cool for a while. The cooling time needed can only be judged as the result of experience. If the section is put into the puddle too early the air in the section expands and many bubbles form. Before the balsam becomes really tacky the section is slid into the puddle, thoroughly coated on one side and then turned over. A circular cover-slip is then dropped on the section and pressed down hard with a cork. This removes all surplus balsam from under the cover slip. When the slide has cooled the solid balsam extruded round the edge of the cover slip can be removed with xylene, and the cover-slip anchored firmly to the slide with ringing cement. This method has the advantage that the section is covered with balsam, which makes invisible any scratches that may have formed on the surface of the section during grinding, yet all the spaces of the lacunae and canaliculi are filled with air, and so stand out with great clarity.

A drawing of each section was made on typing paper with the aid of a camera lucida. The drawing was then cut out and weighed. For purposes of weighing the bone was divided into the categories 'Haversian systems' and 'not Haversian systems'. From a comparison of the weights of the two portions it was possible to determine what proportion of the cylinder was composed of Haversian systems. The change in the relative proportions of 'Haversian systems' to 'not Haversian systems' up and down the length of the bone is gradual, so inaccuracies introduced by the fact that the composition of the bone was measured not at the broken surface but just behind it, were negligible. This was confirmed in several test pieces by taking sections from behind each of the broken surfaces. In all cases the differences found

were within the limits of accuracy of the method of estimation of the proportions of the two histological types. The accuracy of this method was tested by finding the variations produced by treating the same section in the same way several times. The variation was not more than 3 % of the mean. The ultimate strength of the test pieces, in pounds per square inch, was also calculated.

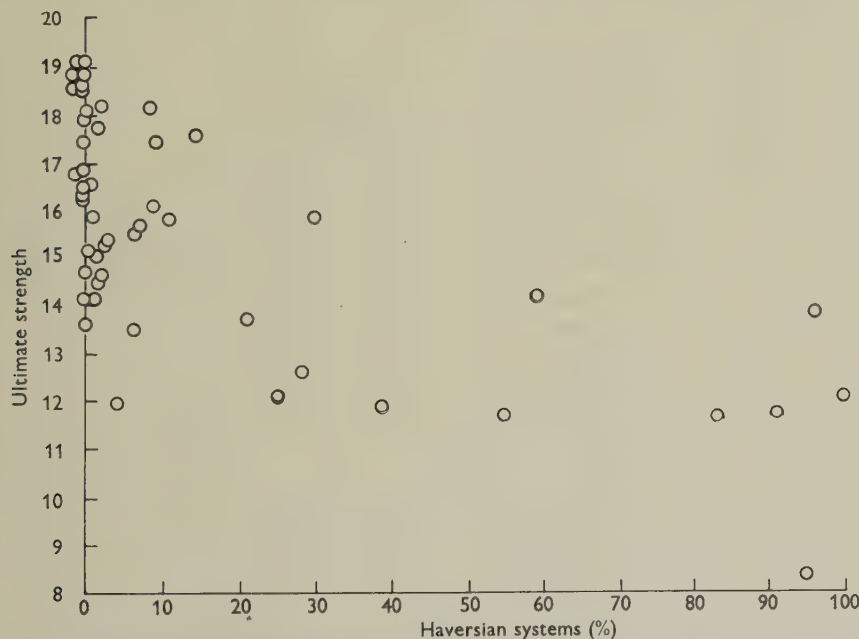
RESULTS

The graph shows the general form of the results (Text-fig. 2). Sixty-four test pieces, coming from four different femora, were tested. Of these fifty were accepted. It is clear that there is a strong negative correlation between the strength of a test piece and the percentage of 'Haversian system' type bone in it.

The general equation for the regression is

$$y = 16,368 - 56.5x,$$

where y is the ultimate strength in pounds per square inch, and x is the percentage of 'Haversian systems' in the test piece. The correlation coefficient, r , for 48 D.F. is 0.6629. This has a probability of very much less than one in a thousand.



Text-fig. 2. Graph showing relation between percentage of 'Haversian system' bone in a test piece and the ultimate tensile strength. Ordinate: ultimate tensile strength in thousands of pounds per square inch.

There is no evidence for heterogeneity between the four bones used, but the number of test pieces taken from each bone was too small to test this point rigorously. In each bone the trend was the same, namely, a relatively large number of samples with few Haversian systems in them and a fairly high ultimate strength, and a few samples with lower ultimate strengths and more Haversian systems.

An attempt was made to determine whether the position of the test piece in the bone had any effect on its strength. It could be argued, quite reasonably, that though there is a correlation between weakness and a high percentage of Haversian systems in the test piece, this by no means makes it certain that it is the high number of Haversian systems that is the cause of the weakness. For instance, it might be that if a certain part of the bone has less than the normal amount of calcium in it, it will be weak, and it might also be true that Haversian systems form in those places in bone that have less than the normal amount of calcium in them. This would produce the same kind of correlation.

Table 1. *Table showing strength of test pieces and the portion of the bone from which they were taken*

Bone	Anterior	Upper		Posterior	Lower		
		Right	Left		Anterior	Left	Posterior
1	16.32	—	—	—	11.64* 15.82*	12.59*	14.19*
2	15.36 16.07* 18.20*	—	14.12	12.10* 17.50	14.64 17.55* 15.80	13.68* 13.89*	—
3	14.10	15.68* 13.54* 15.27	16.80 19.08 18.50	18.55	16.24 18.72	13.64 18.84	8.35* 11.74*
4	18.91 18.11 16.48	19.08 17.41	15.09 14.63 14.57	17.90 14.54* 11.91	18.24 11.88* 15.83	12.02* 11.73*	—
Sum	133.55	80.98	112.79	92.50	156.36	96.44	34.28
N	8	5	7	6	10	7	3
Mean	16.69	16.20	16.11	15.42	15.64	13.78	11.43
	Sum upper quadrants	419.82			Sum lower quadrants	287.08	
		N	26			N	20
		Mean	16.15			Mean	14.35
Excluding all test pieces with more than 5% Haversian systems							
Sum	99.28	51.76	112.79	65.86	99.47	32.48	
N	6	3	7	4	6	2	
Mean	16.55	17.25	16.11	16.46	16.58	16.24	
	Sum upper quadrants	329.69			Sum lower quadrants	131.95	
		N	20			N	8
		Mean	16.48			Mean	16.49

'*' after a figure shows that that test piece contained more than 5% Haversian systems. Four test pieces, from bone no. 1, are excluded because their provenance is uncertain.

The test pieces were arranged according to their provenance, namely, whether they came from the anterior, posterior, left or right quadrants, and whether they came from the upper or lower part of the bone. These measurements are set out in Table 1.

It can be seen that there are differences in the strength of test pieces according to their provenance. Unfortunately, because of the relatively small number of samples, the only difference that can be shown to be significant at the 5% level is the difference between the means for the upper and lower parts. However, some of the other differences are suggestive, especially the difference between the two anterior quadrants. If, however, all those test pieces having more than 5% of Haversian systems in them are ignored, the picture is different. The means for the upper and

lower parts are now similar, as are the means for the two anterior quadrants. All in all it can be said that though there are clear differences in the strength of test pieces coming from different parts of the bone, these differences disappear if all test pieces with more than 5% 'Haversian systems' bone in them are ignored. The provenance of a test piece then, will not of itself determine whether it is likely to be stronger or weaker than the average, *unless* there is some histological difference that marks it off from the average. Conversely, *wherever* a test comes from it is likely to be weaker than the average if it has a high proportion of Haversian systems in it.

DISCUSSION

If then the presence of Haversian systems is associated with weakness in tension, is there any evidence that the weakness is caused by the presence of Haversian systems, or is it necessary to adopt the hypothesis that Haversian systems form in those parts of bone that are already weak?

There are in fact two reasons for supposing that it is the Haversian systems themselves that make the bone weaker: first, they reduce the actual amount of bone present, and secondly, they reduce the total amount of calcium in the bone.

The first effect is probably not very important in young adult animals, but will inevitably have an effect even in them. A Haversian system is formed by the erosion of bone so that a hollow tube is left. Later, lamellae are deposited on the wall of the tube until eventually only the narrow central lumen is left. Figs. 1 and 2, in Pl. 1, show the beginning of this process in a portion of cow's femur in which Haversian systems are encroaching on primary bone from top to bottom. Pl. 1, fig. 1, is an ordinary photomicrograph, and Pl. 1, fig. 2, is a photograph of the corresponding microradiograph. Mature Haversian systems can be seen at the top of the figure. Three immature Haversian systems can be seen in the process of forming, one in the middle of the figure and two on the left. In Pl. 1, fig. 2, they appear as very dark spaces (the light patch in the middle of the topmost cavity is an artefact). In each system only one lamella has yet been laid down. It follows that while Haversian systems are forming a certain amount of the internal volume of the bone will be occupied not with bone substance but with blood and other weak materials. In the test piece, a portion of which is seen in Pl. 1, figs. 1 and 2, a little over 3.25% of the area of the section is occupied by the enlarged lumina of immature Haversian systems. A certain amount of this space will remain when the Haversian systems mature, but nevertheless it is reasonable to say that in bone that is being reconstructed at a fairly high rate at least 3% of the volume of the whole will be occupied not by bone matrix but by the cavities of immature Haversian systems. In senile bone the occupation of the bone volume by enlarged Haversian canals is a characteristic feature, and is caused by the process of erosion taking place at a slightly higher rate than the process of redeposition (Amprino & Bairati, 1936).

The second effect of Haversian systems is probably more important. When the organic matrix of bone is laid down it is quickly impregnated with bone mineral (Vincent, 1955). The amount of mineral laid down is not, however, as great as is present in the surrounding bone. Usually about 60–70% is laid down initially and the rest is added gradually. Amprino (1952) has figured some very interesting histo-

grams showing the percentage calcification of Haversian systems in men and animals of various ages. Using the data supplied by Amprino, and assuming that in adult male human beings 50 % of all bone is Haversian bone (almost certainly an underestimate) I calculated that the skeleton of a youth of 20 years of age was only 95.25 % fully calcified, and that of a man of 76 years of age only 96.5 %. Now it is certain that most of the strength of bone comes from its mineral portion (Hülsen, 1896), and a consideration of the very high specific gravity of the mineral portion of bone makes it most unlikely that the percentage of mineral in the bone would be greater than the optimum value for strength/weight. Therefore any reduction of this value will make the bone weaker volume for volume, and almost certainly weaker weight for weight.

It is interesting to compare the microradiographs of primary osteones with those of Haversian systems. Pl. 1, fig. 3, shows a portion of a cow's femur in which there are both primary osteones, in the upper left corner, and Haversian systems, at the bottom. Superficially the two look somewhat similar, but it can be seen from the corresponding radiograph (Pl. 1, fig. 4) that the primary osteones are as highly mineralized as the surrounding bone, while the Haversian systems are more radio-transparent, and appear darker, which indicates that they have a lower degree of mineralization.

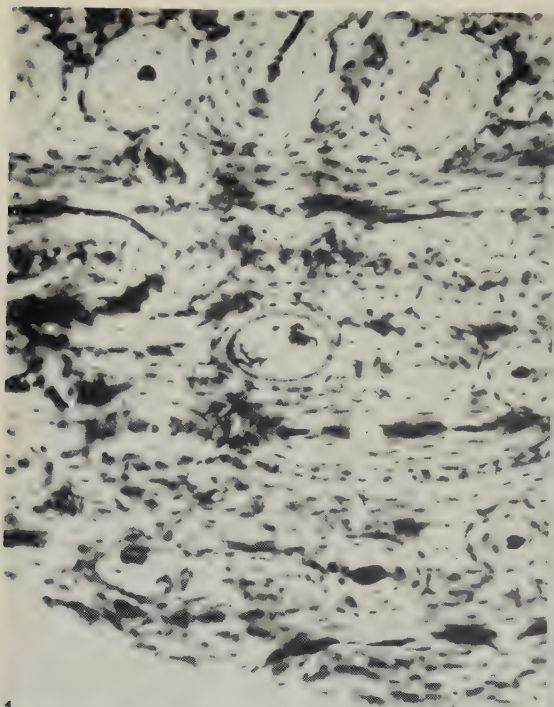
Therefore, for the purposes of the experiments described in this paper it was probably legitimate to lump all 'not Haversian systems' bone together. This is born out by the fact that had the primary osteones been considered equivalent to Haversian systems there would have been no correlation between histological structure and strength in tension, because many of the strongest test pieces had a high percentage of primary osteones.

It seems then that the presence of Haversian systems in bone weakens it, at least in resistance to tension, which is probably the most significant stress biologically. I hope in further papers to discuss other aspects of the significance of Haversian systems.

SUMMARY

1. Test pieces from the diaphyses of femora of oxen were tested for ultimate tensile strength.
2. There was strong negative correlation between the amount of reconstruction that had occurred in a piece of bone, and hence the number of Haversian systems in it, and the tensile strength.
3. Two complementary explanations for this result are given. (a) Immature Haversian systems have large central cavities which reduce the actual amount of bone substance present per unit volume. (b) Newly formed Haversian systems are not as fully mineralized as, and are therefore presumably weaker than, the surrounding primary bone.

I should like to thank Mr H. K. Pusey, Dr A. J. Cain and Mr B. C. Clarke for their helpful criticism. I should also like to thank Professor A. Thom for permission to use the Hounsfield tensometer in the University Engineering Department. This work was carried out while I held a grant from the Medical Research Council.



CURREY—DIFFERENCES IN THE TENSILE STRENGTH OF BONE

(Facing p. 95)

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EXPLANATION OF PLATE

- Fig. 1. Photomicrograph of section of femur of cow showing three immature Haversian systems.
- Fig. 2. Microradiograph corresponding to Fig. 1.
- Fig. 3. Photomicrograph of section of femur of cow showing osteones and Haversian systems.
- Fig. 4. Microradiograph corresponding to Fig. 3.

THE ROUTE OF PERIPHERAL LYMPH TO THE BLOOD STREAM. AN X-RAY STUDY OF THE BARRIER THEORY

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Opinion is divided as to whether the lymph leaving a region can reach the blood stream without passing through a lymph node. Most text-books and reference works of recent date express the opinion formulated by Bartels (1909) that lymph from peripheral regions does not reach the blood without passing through at least one lymph node. In the older literature, however, observations have been reported which indicate that this may not always be correct. Baum (1918) found in dogs that the lymph vessels from the kidneys, adrenals, thyroid and testes could empty directly into the venous system in one-quarter to two-thirds of the cases examined. Similarly, Caylor, Schlotthauer & Pemberton (1927) found that lymph from the posterior poles of the thyroid gland in dogs entered the blood without passing through lymph nodes in two-thirds of the cases. Marhorner, Caylor, Schlotthauer & Pemberton (1927) reported that the same observation was made in two of twenty humans examined. Hellman (1930) states that these isolated exceptions do not disprove Bartels's theory which must be regarded as generally valid, and Yoffey & Courtice (1956) say that, though they themselves never have seen an afferent lymph vessel by-pass a node, 'it is obviously difficult to establish a universal negative. It can, however, be stated with a fair degree of confidence that the by-passing of a node is a most infrequent occurrence.'

As this problem seems to require further investigation it has been examined in more detail.

PERSONAL INVESTIGATIONS

The following questions were investigated:

- (1) In healthy, adult rats is it possible for the lymph leaving a region to enter the blood stream without passing through a lymph node?
- (2) Do various organs, such as testes and extremities, differ in this respect?
- (3) Can the lymph from the testes in dogs reach the venous system without passing through a lymph node?

MATERIAL AND METHODS

The material consists of 117 adult, healthy male rats of two different hooded strains designated A and B, and an albino strain designated C. All of them have been bred as closed populations for many years. Furthermore, the material includes four large dogs.

Earlier investigations were made by injecting various dyes. The findings were

difficult to verify with this method, so a different one has been employed. After killing the animals an X-ray contrast substance was injected in the lymph vessels as described by Kinmonth (1954), or in a lymph node as described by Bruun & Engeset (1956). Radiographs are made of the animals and the findings controlled by dissection under the microscope. The contrast substance employed was metallic mercury which has the advantages of affording excellent contrast, it does not diffuse through the walls of the vessels, it fills very small vessels and the vessels containing mercury are readily found in dissection.

Because of the strong cohesive force of mercury it does not pass readily through lymph nodes and thus affords ideal conditions for by-passing the glands through anastomoses if these are present and of sufficient calibre. Furthermore, the mercury may be 'milked' upward in the vessels during dissection. When the mercury was blocked at the entrance to a node it was possible in this way to try to demonstrate the presence of anastomoses around the gland.

RESULTS

The lymphatic system in rats has been investigated by Job (1915, 1918, 1922-3). His nomenclature is employed in the following descriptions.

Series 1. Twenty-six rats of strain A were injected in the right knee node after exposure of the node without injuring the vessels. About 0.10 ml. mercury was injected in the course of 5-10 min. In all of these animals the contrast went from the knee node to the lumbar node on the same side. In some of them it went further to the renal nodes on the same or opposite side. No anastomoses could be demonstrated which by-passed the lumbar node, either on the radiograph or by dissection. In one rat anastomoses were demonstrated between the knee node and the axillar nodes (Pl. 1, fig. 1). *Conclusion:* after injection of mercury in the knee node, it cannot reach the venous system or the thoracic duct without passing through at least one proximally located node.

It is possible that one node will always empty to a superior node, while peripheral lymph might be able to by-pass the nodes and enter the venous system. In a new series of rats the injections were, therefore, made directly into a peripheral lymph vessel.

Series 2. Twenty-six rats of strain A were injected in one of the subcutaneous lymph vessels on the dorso-lateral side of the foot of the right hindleg. In all these animals the contrast went to and through the knee node, and in all except one, where the vessel ruptured, it went on to the lumbar node on the same side. In some of the animals it progressed further to the renal nodes or to the thoracic duct. In another animal not included in the material, mercury injected into the right hind foot went through fine, subcutaneous vessels to the inguinal nodes and thence to the axillar nodes (Pl. 1, fig. 2). *Conclusion:* after injection of mercury into peripheral lymph vessels it cannot reach the venous system or the thoracic duct without passing through at least two lymph nodes.

It was then investigated whether the result would be the same when mercury was injected into lymph vessels from the testis.

Series 3. Twenty-six animals, strain A, were injected in one of the lymph vessels from the upper pole of the right testis running along the blood vessels in the funiculus, after the testis had been exposed by opening the scrotum. In seven of

the rats the mercury went directly to the thoracic duct without passing through a lymph node (Pl. 2, fig. 3). In four of them the vessel had a side branch to the ipsilateral lumbar node. In ten animals the contrast went to the renal nodes and in nine to the lumbar node. *Conclusion*: when mercury is injected into a lymph vessel from the upper pole of the right testis it may, in a number of animals, proceed to the thoracic duct without passing through a lymph node.

It was considered possible that this peculiarity of lymph drainage from the testis might be due to an unusual mutant which had occurred in laboratory strain A, and which had become widespread as a result of years of inbreeding. The experiment was therefore repeated on a hooded strain from another laboratory (strain B) and on an albino strain from still another laboratory (strain C).

Series 4. Twenty-six animals of strain B were injected in a lymph vessel from the right testis as in series 3. In eight of the animals the mercury went directly to the thoracic duct without passing any lymph nodes. In three of the animals the vessel branched to the ipsilateral lumbar node. In sixteen animals the contrast went to the renal nodes and in two to the lumbar node. *Conclusion*: in strain B also, mercury injected into the lymph vessel from the right testis may reach the thoracic duct without passing a lymph node in a number of the animals.

The experiment was next performed on albino rats. In order to determine whether it made any difference as to which of the large lymph vessels in the funiculus was injected, the injection in this series was made in all of the larger vessels, usually two to four vessels.

Series 5. Thirteen animals of strain C were injected in all of the large lymph vessels of the funiculus on the right side. Radiograph and dissection revealed that in all of the animals the lymph vessels of the funiculus converged into one vessel before reaching lymph nodes or the thoracic duct (Pl. 2, fig. 4; Pl. 3, fig. 5). In four of the rats the contrast went directly to the thoracic duct without passing through any lymph nodes. In three of them the vessels had side branches to the lumbar node. In five the contrast went to the ipsilateral renal nodes and in four to the lumbar node. *Conclusion*: in a third strain, albino strain C, the mercury also passes from lymph vessels in the funiculus directly to the thoracic duct in a number of the animals. The channel is the same regardless of which of the large vessels of the funiculus is injected.

Series 6. Three police dogs and one rottweiler were injected, immediately after being killed, with 2-2.5 ml. mercury in a lymph vessel from the right testis. In two of them (one police dog and the rottweiler) the mercury went directly to the thoracic duct (Pl. 3, fig. 6). In the two others it reached two and three lymph nodes respectively on the right side of the abdominal aorta. *Conclusion*: lymph from the right testis may reach the thoracic duct without passing through lymph nodes in dogs as well as in rats.

DISCUSSION

It has been shown that the lymph in nineteen of sixty-five rats from three different strains may pass from the testis to the thoracic duct without passing through any lymph nodes. This demonstrates that Bartels's theory does not always hold for the testes, and the exception may be valid for other organs as well.

Direct drainage was also demonstrated in two of four dogs, and it is possible that the same phenomenon may take place in other mammals and in humans. This will be investigated in more detail as the phenomenon is of considerable practical interest in connexion with the frequent metastases to the lungs from testis tumours.

In fifty-two rats it has been shown that lymph from the right hind leg will always pass through at least one lymph node before reaching the venous system. This finding supports Bartels's theory. However, reservation must be made for the possibility that anastomoses which by-pass the glands may be of such small calibre that the mercury cannot enter them. Even if this is true, the main lymph stream will probably pass through the nodes.

It has been demonstrated that lymph from the right hind leg can reach the right axilla, an example of the extensive anastomoses of the lymphatic system.

SUMMARY

X-ray investigations have been made on the lymph drainage from the right testis and the right hind leg after injection of mercury into the lymph vessels and nodes.

1. In nineteen of sixty-five rats the lymph vessel from the testis emptied directly into the thoracic duct without passing through lymph nodes. In the other animals the vessel led either to renal nodes or to the lumbar node. The result was the same regardless of which of the large vessels of the funiculus was injected and it was not specific for any single strain of rats.

2. In two of four dogs the lymph vessel from the right testis led directly to the thoracic duct, in the other two to nodes along the abdominal aorta.

3. The lymph from the right hind leg always passed through at least one node before entering the venous system.

4. Anastomoses were demonstrated between the right hind leg and nodes in the right axilla.

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EXPLANATION OF PLATES

PLATE 1

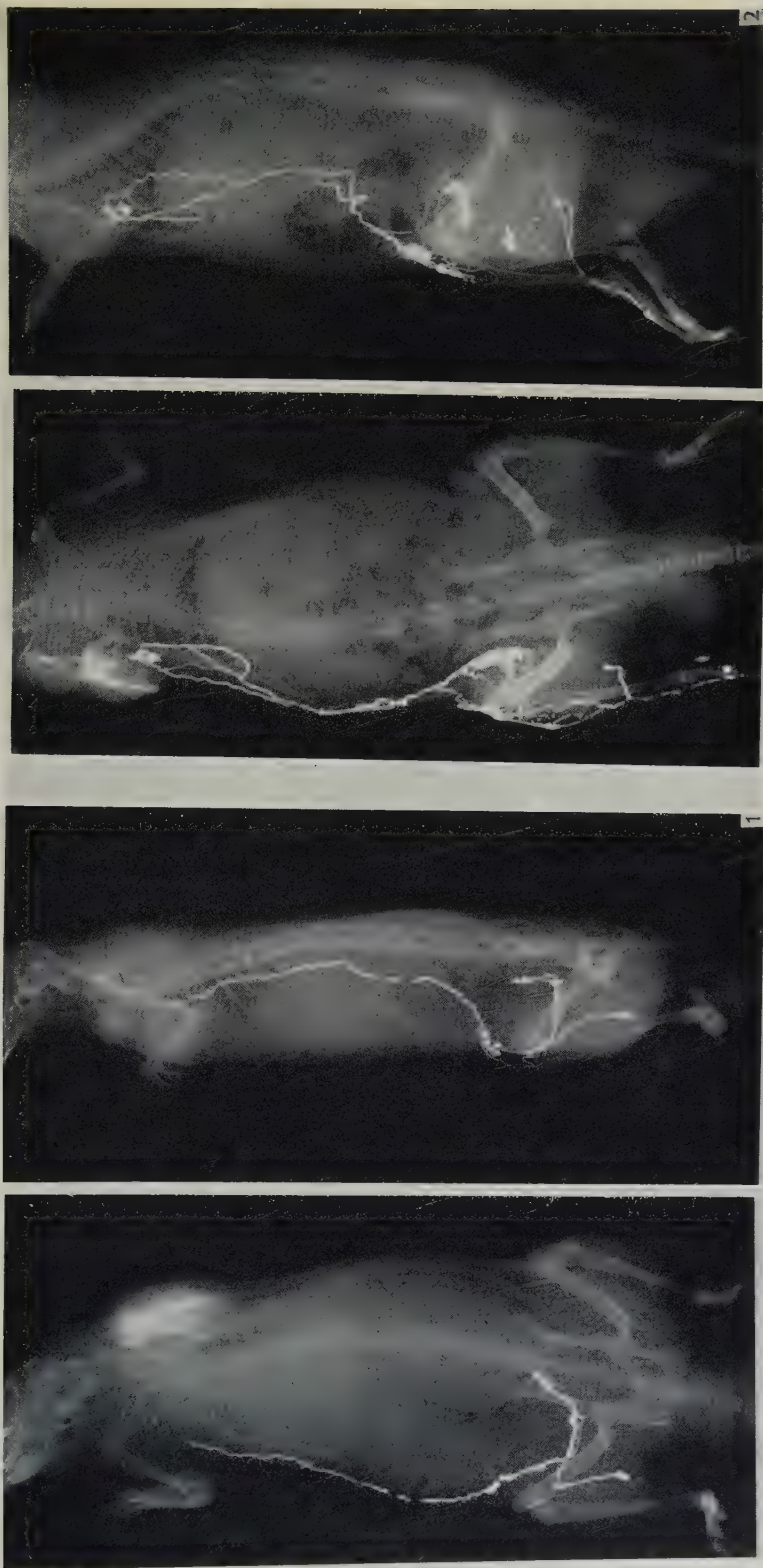
- Fig. 1. Rat. Mercury injected in the right knee node. One branch to the lumbar node, another branch to inguinal nodes and thence to the axilla.
- Fig. 2. Rat. Mercury injected in a lymphatic on the foot. One branch to the knee node. Many small lymphatics to the groin and the inguinal nodes, thence to axillar lymph nodes. Some small vessels in the leg ruptured.

PLATE 2

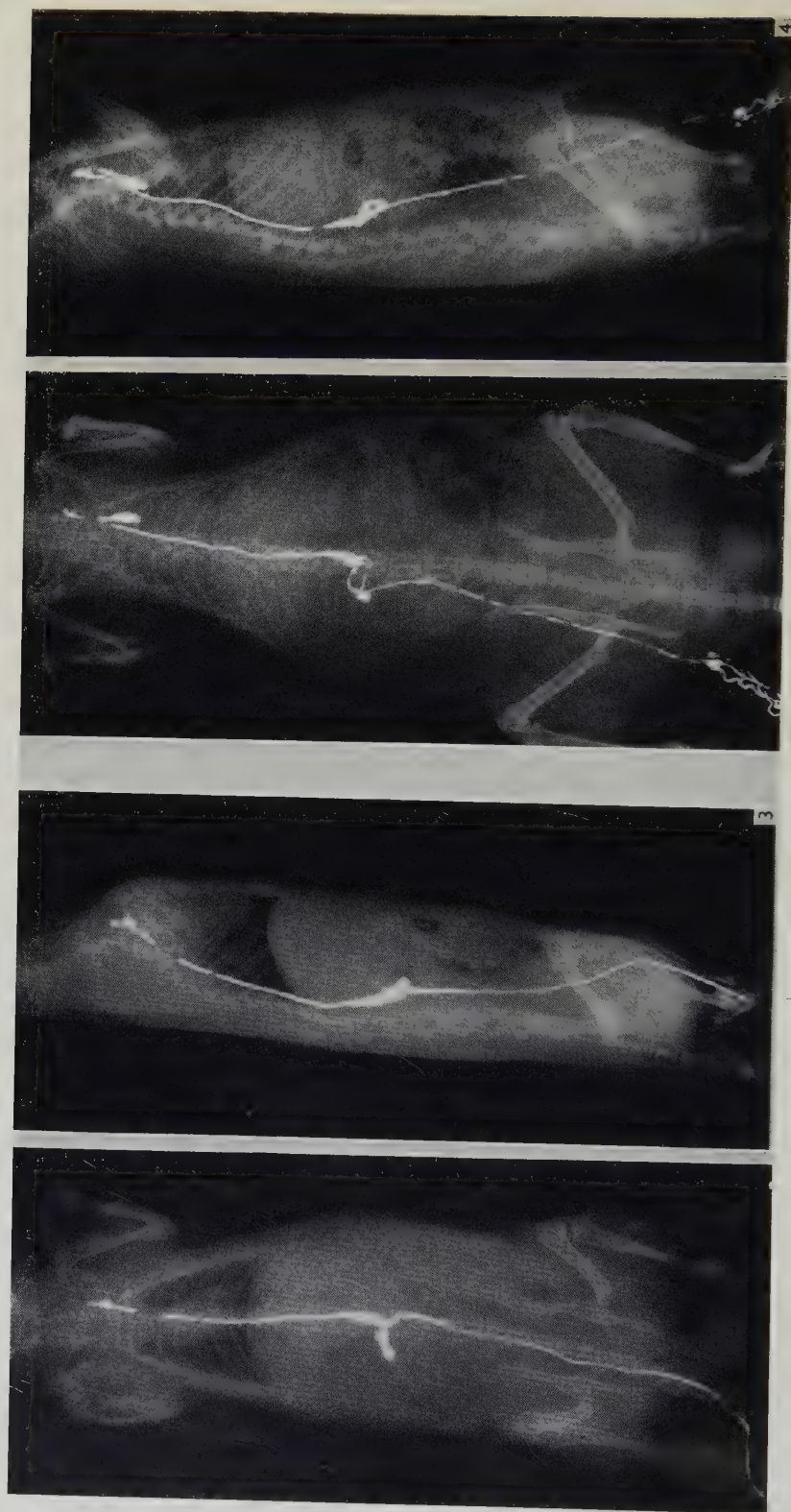
- Fig. 3. Rat. Mercury injected in lymphatic from testis. The lymphatic pathway direct to thoracic duct. In the neck mercury in the vein.
- Fig. 4. Rat. Mercury injected in three lymph vessels of the funiculus. They converge into one vessel before they reach the renal node. From the node branches to thoracic duct.

PLATE 3

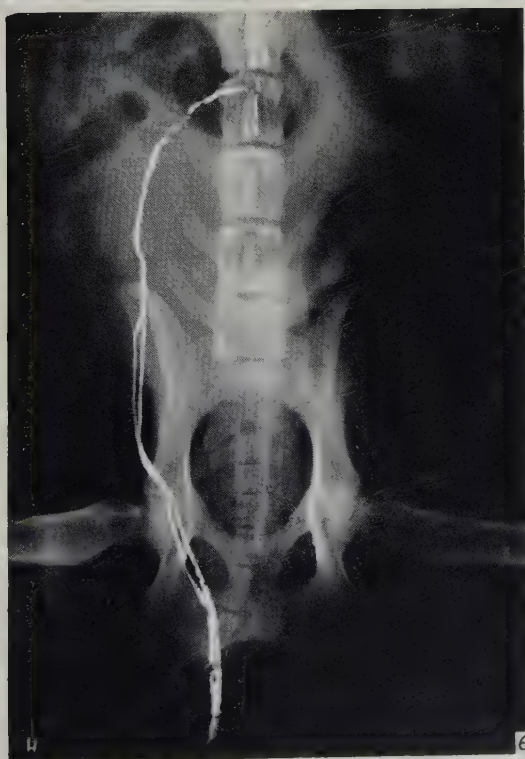
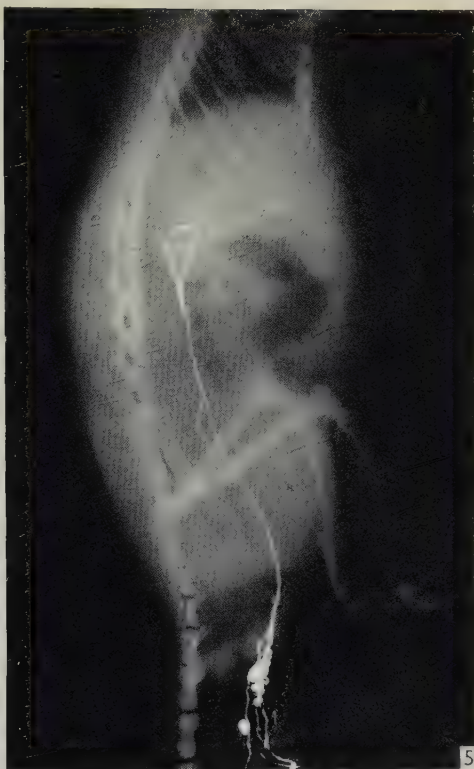
- Fig. 5. Rat. Mercury injected in four lymphatics of the funiculus. They converge before the trunk gives off branches to the lumbar node and two renal nodes.
- Fig. 6. Dog. Mercury injected in a lymphatic from testis. Pathway direct to thoracic duct.



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(Facing p. 100)



ARNFINN ENGESET—THE ROUTE OF PERIPHERAL LYMPH TO THE BLOOD STREAM



INTUSSUSCEPTIVE GROWTH OF SKIN ISLANDS WITHIN WOUNDS

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Billingham & Medawar (1955) showed that intussusceptive growth occurred during the expansion, as healing proceeded, of islands of intact skin left within large skin wounds in rabbits. They ascribed this expansion to tension developed during wound contraction, a term describing the mass inward movement of intact tissue bordering a wound in the early stages of repair. It has been shown that this movement is due to the development of tension within the repair itself (Lindquist, 1946), and it may be mediated by fibroblasts in the granulation tissue (Abercrombie, Flint & James, 1956). It is clear that such tension will be applied to an island of skin left within a wound as well as to the skin bordering it.

Billingham & Medawar found that expanded islands were as thick as normal skin when examined histologically. They did not, however, exclude the possibility that a skin island stretched under tension might change in thickness when removed at autopsy, providing thus a fallacious measurement of its *in situ* dimensions. Two further points appeared of interest. First, in view of the known relative metabolic inertness of collagen (Neuberger, Perrone & Slack, 1951), does collagen deposition keep pace with increase in area? Secondly, is deformation imposed by tension the factor leading to growth, or does stimulation by the adjacent wound play a part? Thus, does the whole skin island participate in the observed expansion, or is its periphery the major contributor?

Skin islands in rabbits have therefore been induced to expand by the contraction of large surrounding wounds. The areas, wet weights, collagen, and nitrogen contents of their central and peripheral parts, distinguished by a tattooing technique, have been determined.

MATERIALS AND METHODS

Adult male rabbits from breeders' stock were used, of mean weight 2.72 kg. (standard deviation 0.45, range 1.71–3.40 kg.). Thirty experimental animals were used, in three groups of ten, autopsied 20, 50 and 100 days after operation.

Animals were anaesthetized with intravenous Nembutal, reinforced as necessary with open ethyl ether. One side of the thorax was shaved with electric clippers, and the skin treated with antiseptic ointment. Animals were tied with both fore- and hindlimbs in extension in a readily reproducible position. A square with sides 5 cm. in length, two of them parallel to the vertebral column, was marked on the thorax with Indian ink. Within, and concentric with it, were marked two further squares whose sides measured 1.5 and 1 cm., respectively, and were parallel to those of the first square. The corners and mid-points of the sides of all three squares were then tattooed with Indian ink, using a No. 22 straight cutting needle held in a small chuck. Excess ink was removed and the animal returned to its cage.

A few days later, after similar anaesthesia, the tattoos were traced on cellophane. Then at aseptic operation skin within the 5 cm. square was removed down to the panniculus, leaving intact a central island bounded by the dots marking the 1.5 cm. square. Excised skin was transferred to a weighing bottle. The tattoos and wound margin were traced on sterile cellophane, the wound treated with penicillin and sulphathiazole powder, dressed with tulle gras and sterile gauze, and a light plaster cast applied. 75,000 units of procaine penicillin were given intramuscularly.

Animals were anaesthetized and redressed in a similar manner 7, 14 and 20 days after operation. By this time no further dressings were needed.

At autopsy animals were killed with intravenous Nembutal, weighed, clipped, and tied as at operation. Tattoos were traced on cellophane, and vertical incisions made down to the panniculus through the tattoos marking the larger of the two central squares. A further tracing of the tattoos was made to record the contraction of the island when freed from the surrounding skin. The island was excised for weighing and subsequent analysis in two parts. The first comprised the innermost and smallest tattooed square, and is referred to subsequently as the 'centre'. The second constituted the remainder of the island, and is referred to as 'periphery'.

Area measurement

From the tracings on cellophane obtained from both types of operation described above, further tracings were made on to paper of standard thickness. These areas were then cut out and weighed, and the results calculated in cm.².

Chemical methods

Skin removed as already described was first weighed. Collagen was brought into solution with 10 % trichloroacetic acid (TCA) at 90° C. for 30 min. (Fitch, Harkness & Harkness, 1955). The suspension was homogenized in a Potter-Elvehjem type homogenizer, heated for a further 30 min. at 90° C., and finally made to a known volume with 10 % TCA. Two 1 ml. volumes of the product were removed for nitrogen estimation by a micro-Kjeldahl method (Ma & Zuazaga, 1942), and the remaining homogenate centrifuged at 2500 r.p.m. for 10 min. Two 1 ml. volumes of the supernatant were removed for hydrolysis and estimation of hydroxyproline by Neuman & Logan's (1950) technique. After colour development absorption measurements were made at 560 m μ (Hulliger, James & Allgöwer, 1957) with a Uvispek absorptiometer, and hydroxyproline contents established from standard curves constructed from known concentrations of hydroxyproline. A factor of 7.46 was used to convert hydroxyproline measurements to collagen.

RESULTS

Animals

Complete results are available for ten animals in the 100-day group, nine in the 50-day group, and for seven animals in the 20-day group. At operation the mean body weights of these groups were 2.47 ± 0.20 , 2.79 ± 0.10 and 2.73 ± 0.13 kg., respectively. Analysis of variance showed that initial body weight did not differ significantly between groups ($F=1.57$, $n_1=2$, $n_2=23$). Over the experimental

periods of 100 and 50 days mean weight increased by $26.9 \pm 5.2\%$ and $13.5 \pm 2.0\%$. In the 20-day group some weight loss was not unexpected in view of the known negative nitrogen balance following trauma. The range of weight change was from -21.7 to $+6.9\%$ (mean $-3.16 \pm 2.56\%$).

Area measurements

Table 1 shows the results of area measurements before and after operation. It is evident that after operation skin elasticity produces expansion of the wound bed and reduction of the island area. The results of analysis of variance, given in the last column, show that wound and island dimensions did not differ significantly between the experimental groups, and that the operative technique was in these respects standard.

Table 1. *Pre- and post-operative areas within wound perimeter, island, and central tattooed square*

		20-day group cm. ² (<i>n</i> =7)	50-day group cm. ² (<i>n</i> =9)	100-day group cm. ² (<i>n</i> =10)	<i>F</i> (<i>n</i> ₁ =2, <i>n</i> ₂ =23)
Wound perimeter	Area pre-operation	24.96 \pm 0.43*	27.34 \pm 1.14	25.91 \pm 0.56	2.21
	Expansion	8.62 \pm 1.55	7.00 \pm 1.10	9.95 \pm 0.75	1.81
Whole island	Area pre-operation	2.16 \pm 0.09	2.24 \pm 0.04	2.35 \pm 0.09	1.39
	Area reduction	0.82 \pm 0.06	0.81 \pm 0.05	0.73 \pm 0.07	0.58
Central tattoo	Area pre-operation	1.04 \pm 0.04	1.00 \pm 0.03	1.11 \pm 0.08	1.00
	Area reduction	0.38 \pm 0.03	0.37 \pm 0.04	0.39 \pm 0.04	0.15
Island periphery	Area pre-operation	1.13 \pm 0.02	1.23 \pm 0.03	1.23 \pm 0.04	2.80
	Area reduction	0.47 \pm 0.06	0.45 \pm 0.03	0.33 \pm 0.05	2.83

* The estimate of variation in this and subsequent tables is the standard error of the mean. 'n' denotes the number of animals from which the mean was derived.

It is thus legitimate to pool island area data to determine whether the central tattooed square and the rim of the skin round it made contributions to reductions in area proportional to their initial dimensions. When pooled, the mean area of the central tattooed square before operation was 1.06 ± 0.03 cm.², and of the remainder of the island (periphery) 1.20 ± 0.02 cm.². When this difference in initial area was eliminated by analysis of covariance, reduction attributable to peripheral and central parts of the island did not differ significantly ($t=0.97$ with 53 D.F.).

From Table 2, island area at autopsy of the 20-day group was 2.69 ± 0.23 cm.², while the pre-operative mean area was 2.16 ± 0.09 cm.². The absolute increase of 0.53 ± 0.22 cm.² is on the borderline of significance at the 0.05% level ($t=2.39$ with 6 D.F.). By 50 days island area had more than doubled. The absolute increases in the 50- and 100-day groups, however, do not differ significantly from each other ($P < 0.05$).

To determine whether the central and peripheral areas of the expanded islands made a proportional contribution to the observed area increase, area measurements were transformed logarithmically. Log area ratios were then determined (i.e. log whole island area minus log central tattoo area). Each group of animals contributed two sets of such ratios, one from measurements made before operation and the other from measurements made before autopsy. The results of analysis of variance

($F=0.62$, $n_1=5$, $n_2=46$) of these ratios demonstrated that they were derived from a statistically homogeneous population, and therefore that expansion was itself homogeneous in the islands.

Comparison of the reduction in island area after operation and after autopsy was made by a similar method. In this case the log area ratios were given by $\log A' - \log a'$, where A' was the area before operation and a' the area after. Similar ratios were

Table 2. *Island areas before and after autopsy*

		20-day group ($n=7$)	50-day group ($n=9$)	100-day group ($n=10$)
Whole island	Pre-autopsy	2.69 ± 0.23	5.48 ± 0.17	6.18 ± 0.39
	Area increase from operation	0.52 ± 0.22	3.24 ± 0.16	3.83 ± 0.38
	Reduction at autopsy*	0.96 ± 0.15	2.27 ± 0.10	2.72 ± 0.28
Central tattoo	Pre-autopsy	1.23 ± 0.11	2.44 ± 0.12	2.79 ± 0.23
	Area increase from operation	0.20 ± 0.11	1.44 ± 0.10	1.68 ± 0.19
	Reduction at autopsy*	0.45 ± 0.08	1.04 ± 0.08	1.29 ± 0.15
Periphery	Pre-autopsy	1.45 ± 0.12	3.03 ± 0.11	3.38 ± 0.24
	Area increase from operation	0.33 ± 0.01	1.80 ± 0.10	2.15 ± 0.23
	Reduction at autopsy*	0.50 ± 0.08	1.23 ± 0.05	1.44 ± 0.19

* Reduction in area when freed from surrounding scar at autopsy.

calculated for the reduction in area after autopsy, and thus again six sets of ratios were provided by the three groups of animals. In this case analysis of variance showed that the log area ratios were not homogeneous ($F=3.81$, $n_1=5$, $n_2=46$). Comparison of ratios derived from operation and from autopsy for each group showed significant differences only in the 100-day group ($t=3.38$ with 18 D.F.), and thus only in this group was the reduction in area at autopsy proportionately smaller to a significant degree than that observed after freeing the island from the surrounding skin at operation.

Wet weight and chemical measurements

Table 3 shows the weights of skin removed at initial operation, and the results of their analysis expressed in terms of unit area (cm^2). The results of analysis of variance shown in the last column again demonstrate homogeneity between the groups. When pooled, the mean values for the composition of skin removed at operation were as follows: weight/ cm^2 114.0 ± 5.6 , collagen/ cm^2 22.4 ± 0.5 and nitrogen/ cm^2 5.3 ± 0.3 mg., collagen accounting thus for about 79 % of total nitrogen and 20 % of wet weight.

Table 3. *Analysis of skin removed at operation*

	20 days ($n=7$)	50 days ($n=9$)	100 days ($n=10$)	F ($n_1=2$, $n_2=23$)
Weight removed (mg.)	2414.7 ± 206.0	3218.1 ± 267.4	2572.7 ± 277.2	2.55
Wt./ cm^2 (mg.)	106.7 ± 9.1	127.4 ± 2.5	107.5 ± 10.9	2.44
Collagen/ cm^2 (mg.)	23.0 ± 2.8	24.6 ± 1.2	20.2 ± 1.7	0.71
N_2 / cm^2 (mg.)	5.1 ± 0.5	6.0 ± 0.5	4.7 ± 0.4	2.34

Measurements of wet weight, collagen, and nitrogen/cm.² made on central and peripheral parts of the expanded islands after autopsy are shown in Table 4, as are the combined (whole island) figures. Variance of means of central and peripheral measurements could clearly arise both from differences between animals and from differences within them. To eliminate the former source of variation individual differences in wet weight, collagen and nitrogen between centre and periphery were calculated in each group of animals and in no case did such mean differences depart significantly from zero. There was thus no evidence that the central and peripheral parts of the island differed in composition in any of the three groups, and it is legitimate to use whole island composition at autopsy for comparison with the composition of the skin removed at operation.

Table 4. *Composition of island at autopsy*

		(mg./cm. ²)		
		20-day (n ₁)	50-day (n ₂)	100-day (n ₃)
Whole island	Wt.	100.3 ± 11.4	116.8 ± 5.6	120.8 ± 3.5
	Collagen	20.7 ± 2.4	24.6 ± 1.6	27.7 ± 2.3
	N ₂	4.7 ± 0.5	5.2 ± 0.3	6.1 ± 0.8
Centre	Wt.	103.7 ± 12.9	105.2 ± 5.9	113.2 ± 12.1
	Collagen	22.0 ± 2.1	23.2 ± 1.7	25.3 ± 2.0
	N ₂	4.9 ± 0.5	4.8 ± 0.3	5.8 ± 0.6
Periphery	Wt.	97.5 ± 10.7	126.2 ± 8.2	127.2 ± 10.4
	Collagen	19.6 ± 2.8	25.8 ± 1.9	29.2 ± 2.5
	N ₂	4.5 ± 0.5	5.5 ± 0.4	6.2 ± 0.4

Such comparisons were made by testing mean individual differences in each group of animals for significant departure from zero. While changes in wet weight per unit area proved not to be significant, collagen per unit area was reduced in the island at 20 days ($0.05 > P > 0.02$), unchanged at 50 days, and increased at 100 days ($0.01 > P > 0.001$) by comparison with skin removed at operation.

While obviously the skin islands remaining after operation could not themselves be analysed, it appears reasonable to assume that their composition did not differ significantly from that of the skin surrounding them. Since the area of the skin removed at operation, its composition, and the island area before operation are known, the probable wet weights, collagen and nitrogen contents of the original skin islands can be calculated and are shown in Table 5, together with the corresponding total values at autopsy.

Table 5. *Wet weight, collagen and nitrogen contents of whole islands at autopsy, with corresponding values for island composition at operation calculated from surrounding skin*

		(All figures are in mg.)			F
		20-day (n=7)	50-day (n=9)	100-day (n=10)	(n ₁ =3, n ₂ =23)
Wet wt.	Initial	229.9 ± 14.8	285.5 ± 17.9	254.4 ± 31.3	1.14
	At autopsy	270.6 ± 33.0	640.9 ± 37.4	739.8 ± 72.3	
Collagen	Initial	49.5 ± 5.8	55.2 ± 8.3	46.7 ± 3.6	0.52
	At autopsy	54.2 ± 5.9	134.1 ± 8.3	172.2 ± 19.0	
Nitrogen	Initial	11.0 ± 0.9	13.4 ± 1.1	11.0 ± 1.2	1.68
	At autopsy	12.5 ± 1.5	28.2 ± 1.5	36.9 ± 3.5	

As was to be expected from the homogeneity between groups of island area before operation and composition of skin removed, initial total values calculated showed no significant difference between groups. (Values of F are shown in Table 5.) Neither wet weight, collagen, nor nitrogen showed significant increases by 20 days ($P < 0.05$ in all cases), and it is clear from the table that the major tissue synthesis occurred between 20 and 50 days after operation.

DISCUSSION

The tattooing technique described had two advantages. First, it enabled central and peripheral parts of the islands to be distinguished and measured. Secondly, it enabled the edges of the expanded island to be separated accurately from the surrounding scar, otherwise a matter of some difficulty, especially in the later stages of repair. Although such tattoos may change slightly in area, there is little doubt that their centres, through which measurements were taken, are stable (Abercrombie & James, 1957). The validity of the tracing method for area measurement has been discussed by Hamlyn (1954). The chemical methods employed have been widely used, and there is no reason to doubt their reliability.

At operation the area of the wound bed increased, and the area of the island left within it diminished homogeneously, both central and peripheral parts reducing in proportion to their original area. The area changes are clearly attributable to the inherent elasticity of the skin. Although Dick (1951) suggested that the elasticity of human cadaver skin could be correlated with its elastin content as observed histologically, water (Jochims, 1934), collagen net work architecture (Rollhäuser, 1950) and ground substance (Ma & Cowdrey, 1950) may also be involved in the mechanism of skin elasticity.

In the group autopsied at 20 days the islands had more than achieved their original area, but the most marked expansion occurred between 20 and 50 days, as evidenced by the lack of significant difference between the absolute increments in area observed in the 50- and 100-day groups. Such a time course of expansion is not unexpected, since tension established by the contracted wound must gradually diminish as skin growth proceeds. Island expansion, like island area reduction after operation, was homogeneous, affecting alike periphery and centre. Tension applied to the island perimeter is likely to be transmitted throughout its substance and no evidence emerges from the area data that the peripheral part of the island, adjacent to an active site of repair, is more responsive to tension than the remainder.

It is perhaps surprising that at no time did the reduction in area of the expanded islands at autopsy exceed that to be expected were they composed of normal skin. Provided that elasticity did not itself change, area reduction presumably reflected tension applied to the islands *in situ*, and thus an island growing in response to tension might be expected to contract more than normal skin when separated from its surroundings. The explanation probably lies in the times chosen for autopsy. At 20 days island area exceeded only slightly its pre-operative value, and if the mechanism of elasticity remained unaltered no difference in area reduction was to be expected. Between 20 and 50 days area more than doubled, while from 50 to 100 days it did not significantly increase. It seems likely that the growth curve fell

off from 20 to 50 days, and that evidence of increased tension should therefore be sought in the earlier part of this period.

The 100-day group was apparently anomalous in that at autopsy the reduction in island area was smaller in proportion than that at operation. This observation may reflect a decrease of elasticity with increasing age (e.g. Dick, 1951).

Nitrogen constituted 4.65 % of wet weight of skin removed at operation, and of it about 78 % was collagenous nitrogen. In these respects rabbit skin resembles that of man. Eisele & Eichelberger (1945) found wet, fat free, human skin to contain 3.37 % of nitrogen, of which 74 % was collagenous.

Measurements of wet weight and nitrogen per cm.² in skin islands at autopsy largely support the histological observations of Billingham & Medawar. Wet weight and nitrogen/cm.² did not differ significantly at 20, 50 or 100 days from the values found for skin removed at operation. This was true both for island centre and for island periphery. As with the area data, no evidence was found to suggest that the synthetic activity of the periphery was increased by the adjacent wound. Broadly it must be concluded that tissue synthesis proceeded uniformly throughout the islands *pari passu* with increase in area, and skin thickness was thus maintained.

The exception, not unexpected, lay in the time course of collagen deposition. Isotope studies (e.g. Neuberger *et al.* 1951; Neuberger & Slack, 1953) have shown collagen to be relatively inert metabolically in comparison with other tissue proteins, although it is sometimes rapidly laid down and sometimes rapidly resorbed. Rapid deposition is seen, for example, in repairing wounds of skin (Abercrombie *et al.* 1954), and rapid resorption in the involuting uterus (Harkness & Harkness, 1954; Harkness & Moralee, 1956). Its inertia is perhaps best described as facultative (Harkness, Harkness & James, 1958).

In the experiments described here collagen, unlike wet weight and nitrogen, showed a significant diminution per unit area in the 20-day islands when compared with skin removed at operation. That this was not an example of 'degrowth' in response to diminished tension in the early stages of repair is suggested by the fact that the total collagen content of the 2-day island did not differ significantly from its estimated value at operation. It appears rather that collagen synthesis lagged behind area, wet weight, and nitrogen changes in the early stages of island expansion.

By 50 days island composition was indistinguishable from that of skin removed at operation, and total collagen, nitrogen and wet weight had more than doubled. Between 50 and 100 days the only significant change was an increase in collagen per unit area, and it is likely that this reflects increasing collagenization of skin with age (Dick, 1951).

The results of the experiments described here thus demonstrate that, with the single exception of a lag in collagen deposition in the early stages, a skin island expanded by the contraction of a surrounding wound maintains its composition. Billingham & Medawar (1955) inferred from histological observation that new collagen was laid down upon the pre-existing framework of the old, but the mechanism of skin growth under these circumstances remains obscure. It is clear that in some way tension stimulates the formation by fibrocytes of extracellular material,

but there is little in the literature to suggest how this result is achieved. A comparable response is perhaps seen in the role played by tension in tendon maturation (Lange, 1929).

Tension applied to the collagen network within which the dermal fibrocytes lie is likely to subject them to local pressure changes and to deformation. An hypothesis perhaps worthy of further investigation is that such changes may influence cellular metabolic activities. It is not without interest that O_2 consumption in muscle is increased by stretch (Feng, 1932) as is Na efflux (Harris, 1954). Unfortunately, there appear to be no references in the literature to comparable phenomena in fibrocytes.

SUMMARY

1. To test the effect of tension on the growth of skin and on its composition, skin islands were left within large cutaneous wounds in rabbits, and induced to expand by the tension imposed as the surrounding wounds contracted.

2. The central and peripheral parts of such islands, delimited by tattooing, have been weighed, and their collagen and nitrogen contents estimated, 20, 50 and 100 days after operation.

3. Islands expanded uniformly in all groups of animals, central and peripheral parts contributing in proportion to their original area, and no differences in composition between centre and periphery were found.

4. Wet weight/cm.² and total N₂/cm.² in no case differed significantly from skin removed at operation, but island collagen/cm.² was significantly reduced at 20 days and significantly increased at 100 days.

5. Total island collagen, wet weight and nitrogen more than doubled in the 20- to 50-day period.

6. It is concluded that tension induced uniform growth throughout the islands, and that the synthetic activity of the periphery was unaffected by the adjacent wound. In the initial stages of expansion, however, collagen increased less rapidly than either wet weight or nitrogen. The hypothesis that cell deformation induced by tension may influence cell metabolism is worthy of further investigation.

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THE SIGNIFICANCE OF THE 'PERICHONDRIAL ZONE' IN A DEVELOPING LONG BONE OF THE RAT

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INTRODUCTION

Ranvier (1873, 1875) described a groove ('encoche d'ossification') in the cartilage at the ends of a growing mammalian long bone which was situated near to its junction with the shaft. Subsequent authors have often referred to Ranvier's ossification groove, but as will be shown later, a phrase of more general application is required, and for this reason the term 'perichondrial zone' will be introduced and employed in this paper. In previous accounts of an ossification groove there have been no descriptions of its early development, nor of the changes occurring in the groove during growth; and what is more surprising, there have been no attempts to define previously what is meant by such a groove.

Some of Ranvier's findings were denied by Schäfer (1878, 1912), who gave a detailed account of an ossification groove. Kollarth (1932), Dahl (1936) and van Wel (1954) described ossification grooves in growing postnatal mammals, but ignored the fibrous architecture and the arrangement of the cells within them. Bausenhardt (1950) described the fibres of a groove as seen by dissection and with polarized light. Langenskiöld (1947) showed how findings in chondrodysplasia may throw light on the normal growth changes occurring in a groove. Langenskiöld & Edgren (1949 *a, b*) made similar deductions from the results of local X-ray injury to the epiphysal plate. Lacroix (1949) described a groove in the postnatal rabbit, and summarized his findings of earlier experiments in which he had observed the formation of a groove in transplants of epiphysal cartilage.

The groove is of considerable interest for the connective tissue within it forms a transitional zone between areas of osteogenesis and chondrogenesis. Our knowledge of the growth changes occurring in this region are far from clearly understood, for it is not readily accessible to the experimental methods that are generally used in the study of bone growth. There are several problems which are often ignored: for example, the periosteum is usually described as being inserted in this region, but there is no precise knowledge on this point. The question of appositional growth at the surface of the epiphysal cartilage and epiphysal plate is unsettled. The mode of formation of the extremities of the perichondrial bony collar, and the origin of the extremities of the periosteal bony cylinder, are still debated.

In order to interpret the structure of a groove, it was decided to study the development of the region with special reference to the formation of fibres and their incorporation into specific structures such as bone and cartilage matrix, periosteum, perichondrium, and ligamentous and tendonous attachments. The long bones of the rat proved very suitable in view of their rapid growth and convenient size. The present description is limited to foetal and newborn animals.

METHODS

Fifteen foetuses of the white rat of known age were obtained from 16 days to birth. The day of finding sperms in the vaginal smear was taken to be day one. The material was fixed in 5% neutral formal-saline, and decalcified in a 10% aqueous solution of the disodium salt of ethylene-diamine-tetra-acetic acid. The hindlimbs were serially sectioned, and stained with Delafield's haematoxylin and eosin, iron haematoxylin and van Gieson, Toluidin blue, or a modification of Long's silver impregnation in which the final stages were omitted.

OBSERVATIONS

(a) *On the form and situation of the perichondrial zone*

An examination of the long bones of the hindlimb in a 20-day-old foetus shows that a groove is only seen in certain situations. Thus the presence of a groove is not an essential feature in a growing long bone. Furthermore, a groove can be confined to only one aspect of a cartilage, which contradicts Ranvier's conception of a circular furrow surrounding the cartilage. Sites of well-marked grooves include the proximal tibial cartilage (Pl. 1, fig. 2), and the anterior aspects of the distal femoral and tibial cartilages (Pl. 1, figs. 3, 4). If these grooves are examined certain features are found which are common to all of them.

(1) The groove lies at the level of the junction of the epiphysial cartilage (where the cells are spherical and irregularly arranged) and the diaphysial cartilage (where, prior to maturation, the cells are flattened and regularly orientated with their long axes lying in the transverse plane).

(2) The groove contains the fibres of the perichondrium which are continuous with the fibres of the periosteum.

(3) The diaphysial border of the groove has a gradual slope as there is an increase in the diameter of the diaphysial cartilage at the level of the junction of the proliferative and hypertrophic zones. The groove is therefore adjacent only to the proliferative zone. It contains the osteoblasts concerned in the formation of perichondrial bone.

(4) The epiphysial border projects sharply from the floor of the groove. Most of the fibres of the perichondrium approach the epiphysial cartilage at right angles and penetrate it for variable distances. This border ends abruptly at the level of the attachment of the most superficial fibres of the perichondrium.

In certain situations there appear to be particularly deep grooves, for example adjacent to the neck of the femur and on the posterior aspect of the distal femoral cartilage (Pl. 1, fig. 3). Their depth is due to the extent to which the epiphysial cartilages project out beyond the diaphysial cartilages. Though these areas satisfy the first three criteria of a typical groove, they differ markedly in respect to the fourth, for the fibres of the perichondrium invariably approach the epiphysis obliquely and do not penetrate its substance. The epiphysial border forms a gradual outward curve and continues beyond the point of attachment of the perichondrium. In view of these differences this type of structure will be referred to as a 'false groove'.

Situations presenting the first three features of a typical groove, but without any projection of the epiphysal cartilage, are found in all the long bones whether a groove is present or not. In such places the perichondrium is similar to that of the false groove, that is the fibres approach the epiphysis obliquely and do not enter its substance. Such 'hemi-grooves' are found adjacent to the greater trochanter (Pl. 1, fig. 1), at the distal and proximal ends of the fibula, and at the posterior aspect of the distal end of the tibia (Pl. 1, fig. 4).

It is felt that the term 'ossification groove' should be confined to those situations where a typical groove is found. It is suggested, however, that a more general term should be used to describe this zone, which would be equally applicable to all similar situations including those where no true groove exists. The advantage of such a terminology is that any further changes in the shape of the epiphysal cartilage during growth would not necessitate changes in phraseology with consequent confusion in description. The term 'perichondrial zone' appears to be particularly suitable, as it can be understood to include both the perichondrium and the perichondrial bone.

(b) On the differentiation of the fibrous structure of the perichondrial zone

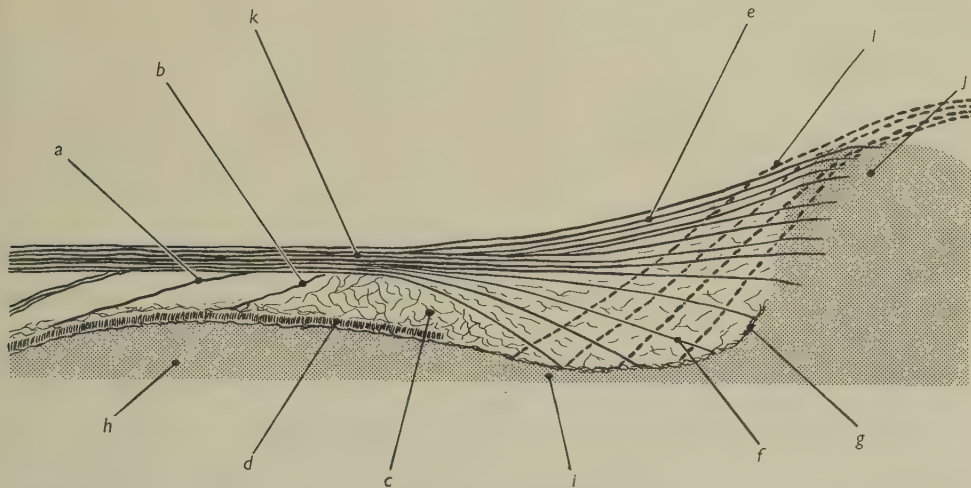
The following account is based on the findings at the distal end of the femur, where anteriorly a typical groove exists, but posteriorly a false groove is formed by the overhanging femoral condyles. The terms periosteum and perichondrium require definition. Both are part of a dense membrane composed of longitudinally running fibres. The term periosteum is used when this is adjacent to bone (i.e. the periosteum surrounds the bony diaphysis), while the term perichondrium is used where this fibrous sheath surrounds the cartilaginous extremity of the bone. It has already been pointed out that in the 20-day-old foetus the extremity of the collar of perichondrial bone lies in the perichondrial zone. Thus the extremities of the perichondrial bone will mark the junction of the periosteum and perichondrium.

In a 17-day-old foetus there is a well-developed sheath of fibres which surrounds the middle of the cartilaginous 'model' of the femur. It is difficult to give a name to this, as it appears before any bone has formed, but nevertheless it marks the zone of preosteoblasts, and therefore it will be referred to as the 'preperiosteum'. The fibres of this structure are coarse, tightly packed, and regularly arranged in a longitudinal direction. At each end it is continuous with the perichondrium. The perichondrium differs in several respects from the preperiosteum. Thus the former membrane consists of a loose irregular network of fine fibres lying in immediate contact with the cartilage, while the preperiosteum is denser, and is separated from the cartilage by the layer of preosteoblasts.

At 18 days the perichondrial bone has appeared and it is possible to define the periosteum. The loose network of fine fibres that previously formed the perichondrium is replaced by coarse longitudinally running fibres. In this and in all later stages it is possible to identify the perichondrium by the loose arrangement of its fibres, in contrast with the fibres of the periosteum which are densely packed.

The epiphysal attachment of the perichondrium is not apparent until the nineteenth day, for the non-articular surface of the epiphysis does not become defined until this stage. It has already been pointed out that the mode of attachment of

the perichondrium to the epiphysis differs in different situations. The perichondrial zone of the anterior aspect of the distal end of the femur has already been defined as a true groove and at 20 days the fibres of the perichondrium may be seen penetrating deeply into the cartilage (Pl. 2, fig. 5), where the fibres appear to break up into bundles of fibrils. Posteriorly the attachment of the perichondrium presents a contrasting picture (Pl. 2, fig. 6). In this situation the fibres of the perichondrium form a superficial network on the surface of the epiphysis, and cannot be traced very far into the cartilage.



Text-fig. 1. A diagram illustrating the arrangement of the fibres of the perichondrial zone as seen in a typical ossification groove. (a) osseous fibre bundle; (b) early osseous fibre bundle; (c) reticulum; (d) perichondrial bone; (e) superficial fibre of bundle of perichondrium; (f) deep fibre bundle of perichondrium; (g) limiting network; (h) hypertrophic zone (of diaphysal cartilage); (i) proliferative zone (of diaphysal cartilage); (j) epiphysal cartilage; (k) periosteum; (l) tendon or ligament fibre bundle. Cartilage stippled. ($\times 400$.)

In contrast to the epiphysis, the diaphysal cartilage is sharply demarked from the perichondrial zone. This is due to a fibrillar network which lies immediately adjacent to the diaphysal cartilage. It is apparent at 17 days, and is continuous with the earliest fibres of the developing perichondrial bone. The later perichondrial bone, which consists of short radially directed fibres (Pratt, 1957), is laid down on this fibrillar network (Text-fig. 1). This 'limiting sheath' is illustrated in Pl. 2, figs. 7 and 8, when it may be seen lying on the apparently fibreless cartilage matrix. A similar appearance was seen in sections which had been incubated with hyaluronidase prior to silver impregnation. This was carried out in order to remove any cartilage ground substance that might be obscuring fibres. These findings suggest that it is a definite fibrous limiting sheath rather than an artefact resulting from the leaching out of ground substance from the most peripheral part of the cartilage matrix.

When at 18 days the perichondrium becomes organized into longitudinal bundles of coarse fibres there is to be seen a more loosely fibred area lying deep to the

perichondrium. This area lies between the limiting network of the cartilaginous diaphysis and the perichondrium, and it is continuous with the subperiosteal space. It contains numerous capillaries and an irregular arrangement of very fine fibres. However, by 20 days this zone has increased considerably in depth and two types of organized bundles of fibres have appeared. First, there are bundles of fibres which blend with the fibrous limiting sheath of the diaphysial cartilage, but do not enter the cartilage matrix (Pl. 2, figs. 7, 8). These pass obliquely towards the middle of the bone and join the fibres of the periosteum (Text-fig. 1). Such fibres form the deep part of the perichondrium, and by the 23rd day are found throughout the zone as far as the commencement of the subperiosteal space (Text-fig. 2). There is, however, in certain situations, another type of fibre bundle; this also is attached to the fibrous limiting sheath, but it passes obliquely towards the epiphysis, that is in the opposite direction to the first type of fibre (Pl. 2, fig. 7). In those situations, where the second type of fibre bundle is seen, a lattice effect results (Text-fig. 1). These fibres that are directed towards the epiphysis are invariably found to be continuous with the fibre bundles of a tendon or ligament, and thus may be interpreted as the fibres of attachment of ligaments or tendons.

Irregularly arranged fine fibre bundles are always to be found between the mature osteoblasts. By the 21st day longitudinally running fibre bundles have appeared in the subperiosteal space of the perichondrial zone, which are continuous with the trabeculae or periosteal bone. These fibres pass obliquely towards the epiphysis and join the fibre bundles of the periosteum. There can be no confusion of these osseous fibre bundles with the deep fibre bundles of the perichondrium, because of the difference in their direction. The osseous fibre bundles must be formed within the subperiosteal space of the perichondrial zone, and their formation must be associated with osteoblasts, as these are the only cells found in close relation to them. Their formation can easily be followed (Text-fig. 1), commencing with the fine reticulum seen between the most distal osteoblasts (Pl. 2, fig. 9), which gradually becomes thicker and gains attachment to the fibres of the periosteum, and finally becomes elongated and reinforced (Pl. 2, fig. 10). There are, of course, the fibres of attachment of tendons or ligaments which present similar appearances to the osseous fibre bundle as all run in the same direction. The tendon and ligament fibre bundles, however, appear much earlier in development, are confined to specific situations and if attached to bone are inserted into the perichondrial bone rather than the periosteal trabeculae. In due course both the ligament and the tendon fibre bundles may become incorporated into trabeculae of periosteal bone.

(c) On the differentiation of the cellular structure of the perichondrial zone

The terminology suggested by Pritchard (1952) will be used in this account of the distal end of the femur. The cells found adjacent to the epiphysial cartilage will be referred to as perichondrial fibroblasts and prechondroblasts, and the cells adjacent to bone as periosteal fibroblasts, preosteoblasts and osteoblasts. There is, however, no accepted terminology for the cells found in the perichondrial zone, and because of the uncertain potentiality of these cells they will be referred to as perichondrial connective tissue cells.

In a 16-day-old embryo the perichondrial connective tissue cells form a closely

packed zone. These cells are irregular in form, have little cytoplasm and a darkly staining nucleus (Pl. 3, figs. 11, 12). This zone merges imperceptibly into the cartilage model. The cells of the cartilage model (chondroblasts), in contrast to the perichondrial connective tissue cells, have pale staining nuclei, more cytoplasm and are separated by dense intercellular substance. The chondroblasts are irregularly arranged in the epiphysis but are orientated along transversely running arcs in the diaphysis, the junction between these two cartilages lies adjacent to the zone of perichondrial connective tissue cells. The perichondrial connective tissue cells are continuous with the surrounding, but more loosely packed, mesenchymal cells. The former are continuous towards the interzone with the prechondroblasts that lie adjacent to the epiphysal cartilage. These latter cells form a layer of closely packed rounded cells separated by fine argyrophilic fibres. Towards the middle of the cartilage model the perichondrial connective tissue cells are continuous with the layer of elongated cells that lie adjacent to the cartilaginous diaphysis. The latter cells will differentiate into the cells of the periosteum.

A rapid increase in the matrix separating the cells of the diaphysal cartilage now occurs, and this leads to a clear demarcation of the cartilage and the perichondrium. The floor of the perichondrial zone becomes defined; and at the same time the curvature of the floor appears in the posterior part of the zone, i.e. on the back of the femur. The perichondrial connective tissue cells remain densely packed anteriorly, and by 19 days they are limited externally by a zone of perichondrial fibroblasts, which are flattened cells with oval nuclei. It is not possible to say whether these fibroblasts have arisen from the perichondrial connective tissue cells, or from the surrounding mesenchyme. In the posterior part of the perichondrial zone (a false groove) there is no evidence of perichondrial fibroblasts and the perichondrial connective tissue cells are very loosely arranged (Pl. 3, fig. 13). Numerous capillaries lie in this loose connective tissue.

Anteriorly a typical ossification groove has differentiated by 20 days, produced by overgrowth of the epiphysal border of the perichondrial zone (Pl. 3, fig. 14). The increase in the depth of the zone of perichondrial connective tissue cells has resulted in a more loosely arranged tissue. However, the external zone of perichondrial fibroblasts is closely packed and its cells, which show numerous mitotic figures, have become very elongated, and are now indistinguishable from the periosteal fibroblasts. The arrangement of the fibres of the perichondrium is no doubt associated with the laminated structure of this tissue. The epiphysal prechondroblasts which are adjacent to the perichondrial connective tissue also have a laminated arrangement.

It should be pointed out that in situations where the perichondrial zone is in the form of a hemigroove, the cellular structure of the latter resembles the early stage of a typical ossification groove where the perichondrial connective tissue is dense, but not laminated, and has an outer layer of perichondrial fibroblasts.

The differentiation of the osteoblasts found in the perichondrial zone requires further comment. At 17 days the preosteoblasts are confined to the middle of the diaphysal cartilage and are separated from the perichondrial connective tissue cells by a zone of closely packed elongated cells. These latter cells appear to differentiate into preosteoblasts, and then into osteoblasts. The osteoblasts gradually approach

the perichondrial zone, reaching the level of the junction of the hypertrophic and proliferative zones of the diaphysial cartilage at 18 days, and attaining their final extent at 20 days, when they reach the level of the middle of the proliferative zone. By this stage the osteoblasts form a deep and closely packed layer, and remain as such until birth.

Mitotic figures are seen in the perichondrial zone amongst the perichondrial connective tissue cells, perichondrial fibroblasts, prechondroblasts, periosteal fibroblasts, and preosteoblasts. Such mitotic figures appear to be randomly distributed, and are more numerous in the early stages than in the late ones. The greatest mitotic activity is seen at 19 days, the significance of which will be discussed later.

DISCUSSION

The structure of the perichondrial zone and the growth of the bone

It has been shown that at the distal end of the femur the differentiation of the perichondrial zone closely follows the differentiation of the epiphysial and diaphysial cartilages. It is to be expected therefore that the growth of the cartilages will also influence the structure of the perichondrial zone, and this requires comment.

It is convenient to consider the growth of the cartilage in two phases. There is an early phase lasting up to the 19th day during which the cartilage becomes increasingly demarcated from the surrounding tissues, and elongation of the bone is largely a result of changes occurring in the middle of the cartilaginous diaphysis. During the later phase, which occurs after the appearance of a medullary cavity, the elongation of the bone results from changes occurring in the diaphysial cartilages, at the extremity of the shaft. The histological details of these processes will be described elsewhere, and in this discussion it will be assumed that the elongation of the diaphysis is chiefly due to the enlargement of its cartilage cells.

During the early phase of growth the cartilaginous boundaries of the perichondrial zone become defined. As the elongation of the diaphysial cartilage occurs principally in its middle portion it means that the cartilaginous boundaries of the perichondrial zone, though migrating, are not elongating to any extent. The over-all elongation of the bone must, however, effect the fibres of the perichondrium, and it is interesting to note that these become organized into fibre bundles about this time. Though little elongation is occurring in the cartilage adjacent to the perichondrial zone there is, however, some increase in width, particularly in the region of the junction of the proliferative and the hypertrophic zones. The differentiation of the perichondrial zone osteoblasts follows the appearance of this 'diaphysial eminence', and the two features remain associated (probably because hypertrophic cartilage induces the differentiation of osteoblasts).

In the later phase of the growth of the distal femoral cartilage the elongation is occurring principally in the region of the junction of the proliferative and the hypertrophic zones of the diaphysial cartilage. Thus the part of the perichondrial zone lying adjacent to the 'diaphysial eminence' will be undergoing considerable elongation and is due to become incorporated into the bony diaphysis. The remaining portion of the perichondrial zone will be relatively static in terms of longitudinal growth, although the circumference will be increasing. It is not unreasonable to

suggest that the cellular contents of the perichondrial zone will follow any growth changes in the adjacent cartilage. Thus the perichondrial connective tissue cells will form a zone which is being stretched transversely, but not elongated, except for the possible influence of the fibres of the perichondrium. In contrast to this the osteoblasts form a zone which will be stretched both transversely and longitudinally.

The interpretation of the fibrous structure of the perichondrial zone

The original account of the structure of an ossification groove by Ranvier (1873) was not illustrated. He described curved fibres ('fibres arciformes') passing from the epiphysial cartilage to the bone of the diaphysis. Two years later (1875) he described these fibres again and used for his illustration the calcaneum of the dog, and showed that these fibres were concave towards the epiphysis. Schäfer (1878) questioned Ranvier's findings and described linear 'longitudinal osteogenic fibres' passing from the epiphysis to the bone of the diaphysis. There is little doubt that these linear fibres correspond to the longitudinally arranged fibres of the perichondrium which have been seen in the rat. Schäfer also observed some other fibres in an ossification groove which crossed the longitudinal fibres, and which he thought might become 'perforating fibres'. Similar fibres have been seen in certain situations in the rat where there is no doubt that they are the fibres of attachment of tendons or ligaments. The direction of these fibres is similar to that of the 'fibres arciformes' described by Ranvier and therefore it is likely that he was observing the attachment of the tendo calcaneus. Lacroix (1949) described an ossification groove in the region of the anterior tubercle of the tibia in a postnatal rabbit, and he distinguishes between the periosteal fibres and the fibres of attachment of the ligamentum patellae.

Ranvier (1873) described the 'fibres arciformes' as being continuous with the matrix of the cartilage. It has been possible to confirm this in both the case of epiphysial attachments of tendons and the epiphysial attachment of the perichondrium. Schäfer (1878), however, claimed it to be exceptional to find the longitudinal fibres penetrating the cartilage. These different findings are explained by the two modes of perichondrial attachment seen in the rat, namely the deeply penetrating parallel fibres, or the more superficial lattice-like arrangement. Dahl (1936) and Policard (1941) believed that the fibres of the perichondrium were formed by the cartilage cells of the epiphysis rather than being preformed and later incorporated into the epiphysis. Neither author, however, described the arrangement of these fibres. The relation of the deep perichondrial fibres and the tendon fibres to the cartilage forming the floor of the perichondrial zone has not been understood in the past. The present investigation shows that they blend with a fibrous limiting network which has not been previously described.

The relation of the fibres of the perichondrial zone to bone matrix

Most if not all of the accounts in the past have assumed that the fibres found in the perichondrial zone will become incorporated in due course into bone matrix. Weidenreich (1930) described longitudinal fibres in the matrix of the perichondrial bone of the human foetal femur which he claimed were continuous with the 'fibres arciformes'. The early perichondrial bone of the rat consists of longitudinally arranged fibre bundles. It was possible to trace these fibres into the perichondrial

zone where they were found to be continuous with the limiting network of the floor of the groove, and thus were not directly continuous with 'fibres arciformes'; likewise the late perichondrial bone had no extraneous fibres incorporated into its matrix unless tendon fibres were attached to the underlying fibre network.

The early embryonic woven bone, which is the only periosteal bone formed up to the 20th day, does not reach the perichondrial zone, and there is no question of adventitious fibres being incorporated into it. The late periosteal bone, consisting of needle-like trabeculae, contains coarse longitudinal fibre bundles. These osseous fibre bundles first appear in the subperiosteal space as a fine fibrous connexion between the perichondrial bone and the deep surface of the periosteum. Later they become elongated and thickened. These changes are to be expected because the bone and periosteum elongate in a different manner. The bone elongates by addition to its extremities in the region of the diaphysial cartilage, while the periosteum elongates equally throughout its length, as shown by the experiments of Warwick & Wiles (1934) and Lacroix (1948). In this connexion it is interesting to note that these needle-like trabeculae do not commence to form until the bone growth is confined to the extremities. It is not possible therefore to accept Schäfer's (1878, 1912) description of the 'longitudinal osteogenic fibres', if by this term he meant the fibres of the perichondrium. For he described these fibres as passing into the substance of the periosteal bone.

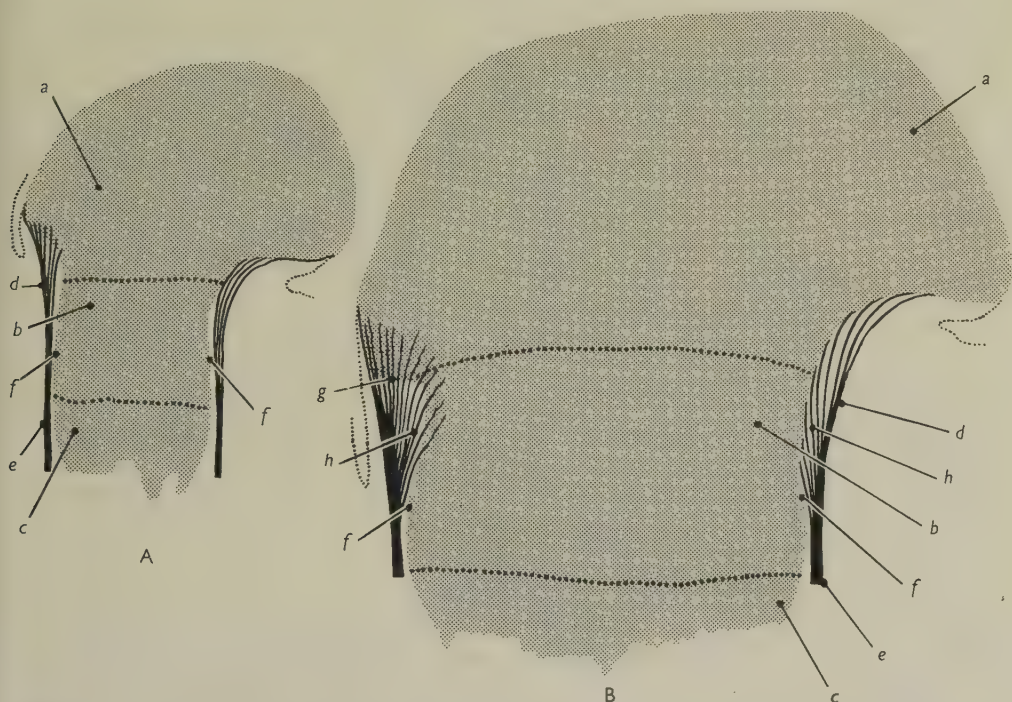
Ranvier (1873, 1875) described the 'fibres arciformes' entering the substance of the periosteal bone. If by 'fibre arciformes' he meant tendon attachments, and by periosteal bone he meant late periosteal bone, then we can agree with these findings. The same comment applies to the accounts of van der Stricht (1889) and Weidenreich (1930). It is significant that all these workers were describing situations where one would expect to find tendon or ligament attachments. Lacroix (1949) pointed out that the state of affairs found in the anterior part of the proximal groove of the rabbit's tibia was unlike that found in other places. Here the fibres of the ligamentum patellae became incorporated into plaques of periosteal bone. Ranvier (1873) described the 'fibres arciformes' as only partially incorporated into bone matrix. This was confirmed by van der Stricht (1889) in the tibia of the calf, but is not seen in the rat, and it may well be a finding that is confined to larger mammals.

The origin and fate of the cells of the perichondrial zone

The perichondrial connective tissue cells have been occasionally described in the past, but their origin has never been considered. Ranvier (1873) described longitudinal rows of cells in his account of an ossification groove. Most subsequent workers have been content with this brief description but Lacroix (1949) described fine connective tissue cells which continued to divide, and van Wel (1954) referred to a loose connective tissue. It has not been appreciated in the past that the cellular arrangement in the perichondrial zone changes with development, and varies from one situation to another.

Schäfer (1878, 1912), Kollath (1932) and Lacroix (1949) believed that the cells found in the ossification groove differentiated into cartilage cells which became incorporated into the epiphysial cartilage. These views were not backed by any substantial observations and have frequently been denied by workers such as Dahl

(1936), who supported the original suggestion of Ranvier (1873, 1875), which was that the cells of the ossification groove were actually derived from the cartilage by a process of chondrolysis. Similar views were held by Langenskiöld (1947) and Langenskiöld & Edgren (1949*a, b*), who thus interpreted the findings in Ollier's disease, and in a similar condition in rabbits induced by local X-ray injury, where a peripheral defect in the epiphysial plate is repaired by growth from within the cartilage. Van Wel (1954), however, believed that the cells found in the ossification groove were neither derived from cartilage cells nor did they give rise to them.



Text-fig. 2. Longitudinal sections through the distal femoral cartilage at 19 days (A) and 23 days (B) showing the changes that occur in the perichondrial zone. Note the migration of the epiphysial lip in the anterior part of the zone (g) and the increase in the deep fibres of the perichondrium (h). ($\times 96$.) (a) epiphysial cartilage; (b) proliferative zone (of diaphysial cartilage); (c) hypertrophic zone (of diaphysial cartilage); (d) perichondrium; (e) periosteum; (f) distal extent of osteoblasts.

The present account shows that in the early stages the perichondrial connective tissue cells pass imperceptibly into the zone of prechondroblasts which surround the epiphysial cartilage, but this in itself does not prove that chondrogenesis is occurring. However, the later incorporation of the perichondrial fibre bundles into the substance of the epiphysial cartilage does suggest that chondrogenesis is taking place. The most convincing evidence in support of chondrogenesis is the changes that occur during the late foetal life in the anterior part of the perichondrial zone at the distal end of the femur (Text-fig. 2). At 19 days the epiphysial border of the anterior part of the perichondrial zone lies at the same level as that of the posterior

part. However by the 23rd day the anterior epiphysial border appears to have migrated towards the centre of the bone and now lies at the level of the junction of the epiphysial and diaphysial cartilages. It would thus appear that this extension of the epiphysial cartilage results from a direct transformation of the perichondrial connective tissue cells into cartilage cells, and this is supported by the progressive incorporation of the perichondrial fibre bundles into the cartilage. It is therefore suggested that appositional cartilage growth occurs by the differentiation of the perichondrial connective tissue cells into cartilage cells and that it is particularly marked in those situations where a typical ossification groove exists.

The question now arises—can the perichondrial connective tissue cells differentiate into osteoblasts? Ranvier (1873, 1875), Langenskiöld (1947) and Langenskiöld & Edgren (1949*a, b*) believed the cells of the ossification groove to be an intermediate stage between chondroblast and osteoblast. Van Wel (1954) believed that these cells were entirely osteoblastic. From what has already been said about the growth processes occurring in the cartilage adjacent to the osteoblastic zone, it would appear that the osteoblasts might soon become exhausted if they are not replaced. There is however no evidence of the former, and at the same time there is little mitotic activity in any part of the zone after 19 days. The perichondrial connective tissue cells become increasingly loosely packed after 19 days, and this is associated with an increase in the capillaries of the area. The former finding could be taken to be that the perichondrial connective tissue cells are migrating into the osteoblastic zone and undergoing differentiation; alternatively the loss of cellular density may be attributed to the increasing depth and circumference of the perichondrial zone. Thus the available evidence suggests that the perichondrial connective tissue cells may differentiate into osteoblasts, but the evidence is not conclusive.

SUMMARY

1. The perichondrial zone is defined in the long bones of the hindlimb of the prenatal rat. It includes Ranvier's ossification groove, and other similar areas.
2. The differentiation of the fibres of the perichondrium at the distal end of the femur is described. Attention is drawn to the differences in the mode of attachment of the fibres of the perichondrium to the epiphysial cartilage.
3. The fibres found in the subperichondrial spaces are: (*a*) the deep fibres of the perichondrium, (*b*) the fibres of attachment of tendons or ligaments, (*c*) fine reticular fibres, and (*d*) those of the limiting network which lines the floor.
4. Coarse longitudinal fibre bundles arise *de novo* in the subperiosteal space and are incorporated into bone.
5. The arrangement of the perichondrial connective tissue cells at the distal end of the femur of the prenatal rat is described, and their differentiation is discussed. There is evidence that chondrogenesis takes place in the perichondrial zone.
6. The structure of the tissues of the perichondrial zone is discussed in relation to growth of the bone. It is suggested that the subperichondrial tissues are little affected by longitudinal growth.

The author is grateful to Professor J. D. Boyd for helpful criticism. He is also indebted to Mr J. W. Cash and Miss C. J. Hitchins for technical assistance and to Mr J. F. Crane for the photography.

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EXPLANATION OF PLATES

Figs. 1-14 show longitudinal sections of the perichondrial zone in the hindlimb of foetal rats. The sections illustrated by figs. 1-10 have been impregnated with silver using an abbreviated Long's method, while figs. 11-14 show sections that have been stained with haematoxylin and eosin.

PLATE 1

The extent of the perichondrial zone is indicated by arrows. (*a*) epiphysal cartilage; (*b*) proliferative zone (diaphysal cartilage); (*c*) hypertrophic zone (diaphysal cartilage); (*d*) perichondrium; (*e*) periosteum; (*f*) perichondrial bone; (*g*) periosteal bone. ($\times 48$.)

Fig. 1. Proximal end of femur (20 days) which shows a hemigroove on both aspects.

Fig. 2. Proximal end of tibia (22 days) which has a typical ossification groove on both aspects. Note the ligaments (*h*) passing into the perichondrial zone.

Fig. 3. Distal end of femur (20 days) which shows a typical ossification groove anteriorly (to the right) and a false groove posteriorly (to the left).

Fig. 4. Distal end of tibia (20 days) which has a typical ossification groove anteriorly (to the right) and a hemigroove posteriorly (to the left).

PLATE 2

Fig. 5. The epiphysal lip of the anterior part of the distal femoral perichondrial zone at 20 days. Note the linear fibre bundles (*a*) of the perichondrium entering the epiphysal cartilage (*b*). ($\times 780$.)

Fig. 6. The epiphysal lip of the posterior part of the distal femoral perichondrial zone at 21 days, showing the relation of the perichondrial fibres (*a*) to the cartilage matrix (*b*) of the epiphysis. ($\times 780$.)

Fig. 7. The floor of the posterior part of the proximal tibial perichondrial zone at 22 days, showing the limiting network (*a*) ensleeving the cartilage of the diaphysis (*b*). Note the decussation of the deep perichondrial fibres (*c*) and the fibres of the posterior cruciate ligament (*d*), both are blending with the limiting network. ($\times 780$.)

Fig. 8. The floor of the posterior part of the proximal tibial perichondrial zone at 22 days, showing the deep perichondrial fibres (*a*) blending with the limiting network (*b*). There are no tendon or ligament fibres in this region. ($\times 780$.)

Fig. 9. The diaphysal extremity of the posterior part of the proximal perichondrial zone of the tibia at 22 days, showing the subperiosteal reticulum (*a*) lying between the osteoblasts. These fibres are in contact with both the fibre bundles of the periosteum (*b*) and the perichondrial bone (*c*). ($\times 780$.)

Fig. 10. The posterior extremity of the bony diaphysis of the tibia at 22 days, showing the coarse osseous fibre bundles (*a*). These bundles are distinct from the fibres of the periosteum (*b*). ($\times 780$.)

PLATE 3

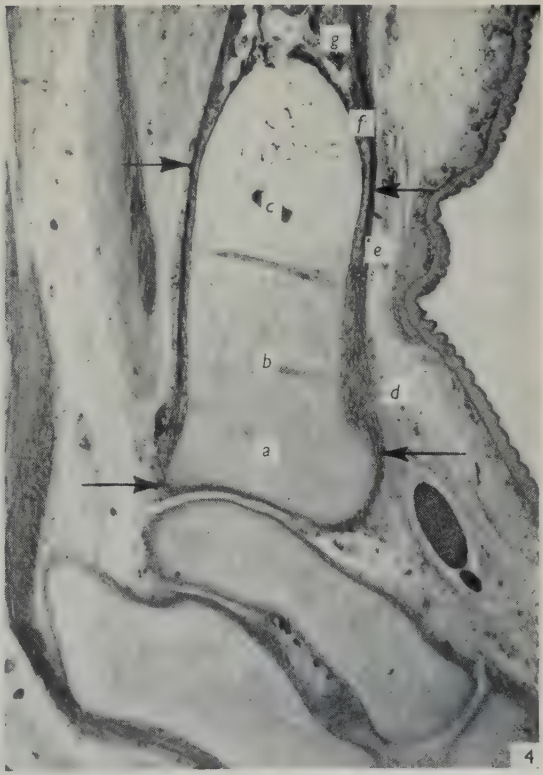
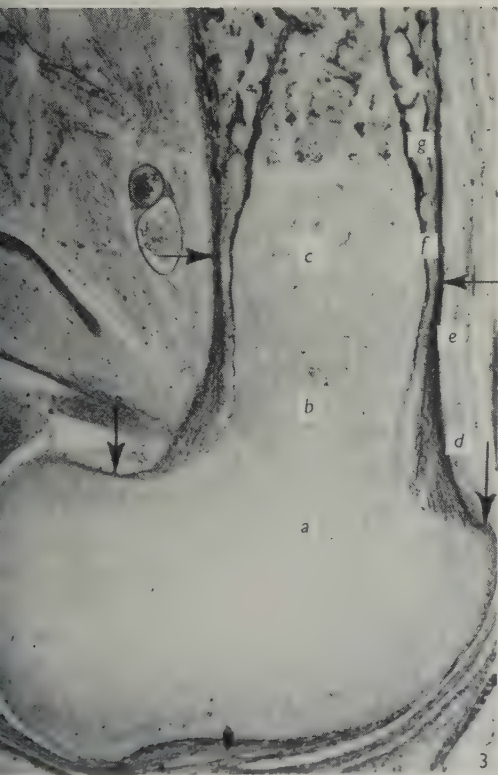
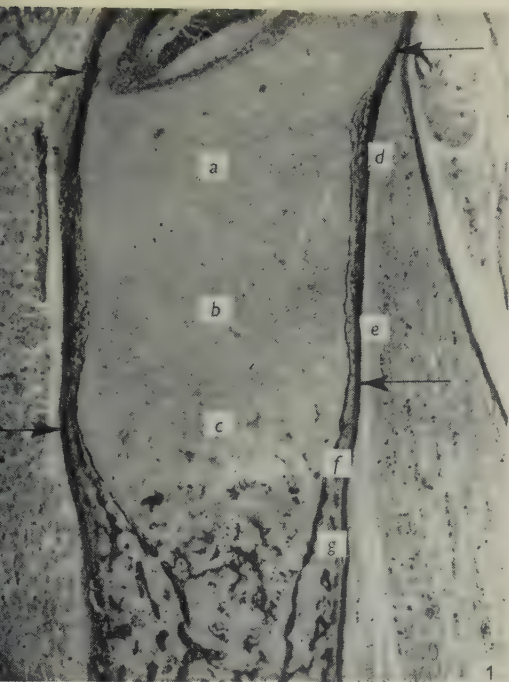
Figs. 11-14 distal end of femur. (*a*) epiphysal cartilage; (*b*) proliferative zone (diaphysal cartilage); (*c*) hypertrophic zone (diaphysal cartilage); (*d*) perichondrial connective tissue cells; (*e*) prechondroblasts; (*f*) perichondrial fibroblasts; (*g*) preosteoblasts; (*h*) osteoblasts; (*i*) periosteal fibroblasts; (*j*) capillary.

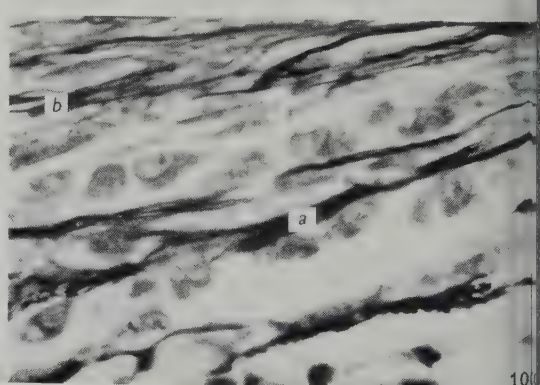
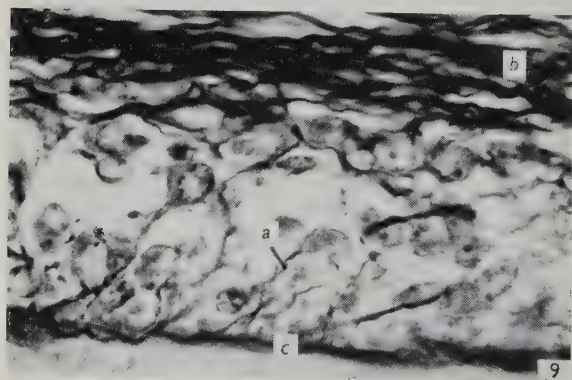
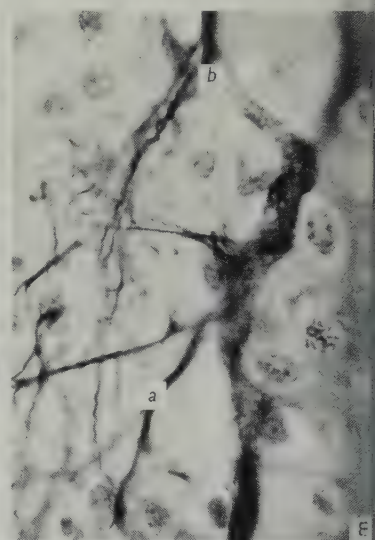
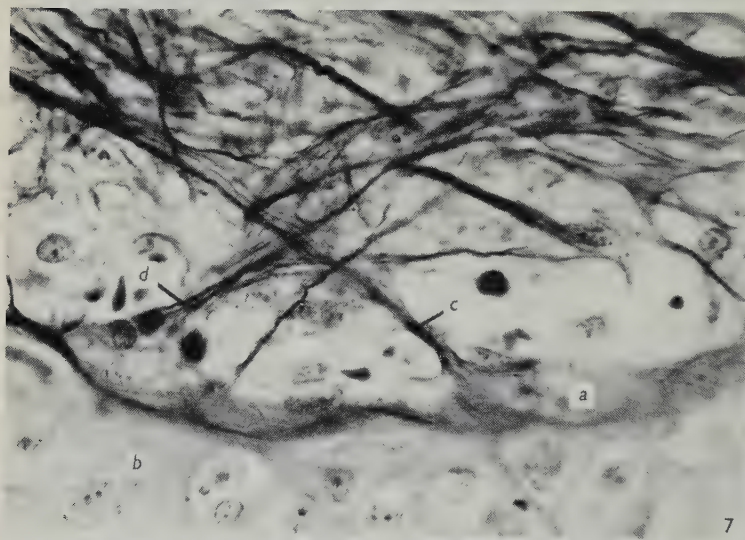
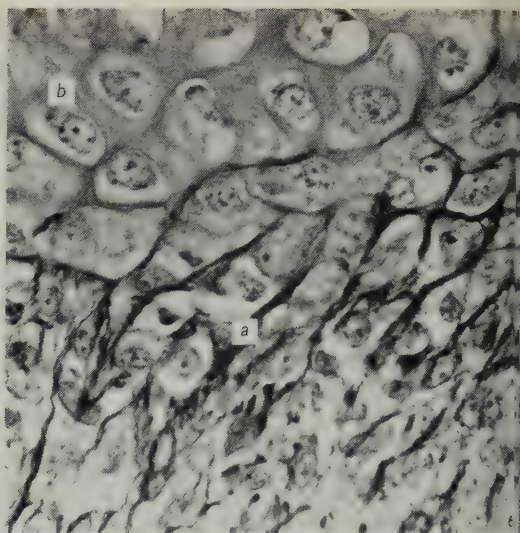
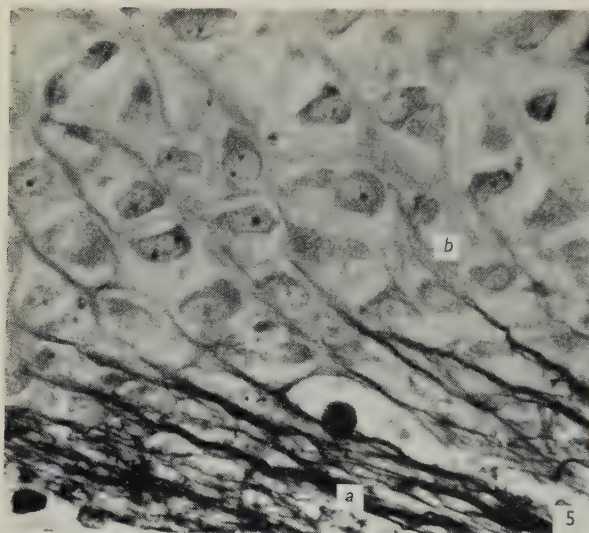
Fig. 11. Anterior part of the perichondrial zone (16 days). Note the absence of a distinct floor to the zone in this figure and in fig. 12. ($\times 375$.)

Fig. 12. Posterior part of the perichondrial zone (16 days). ($\times 375$.)

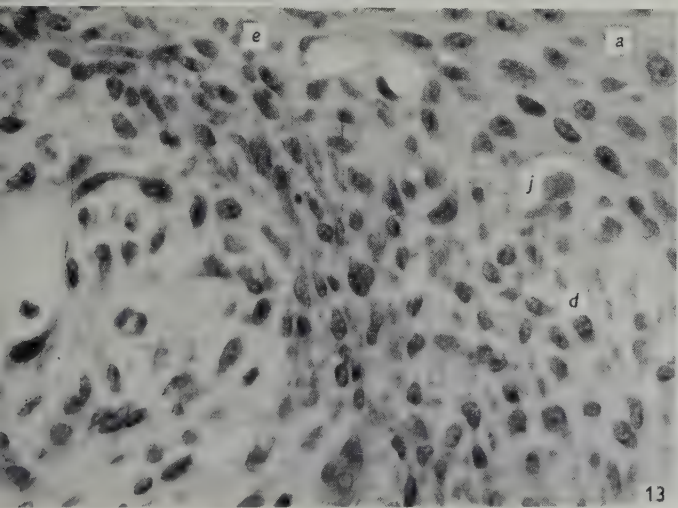
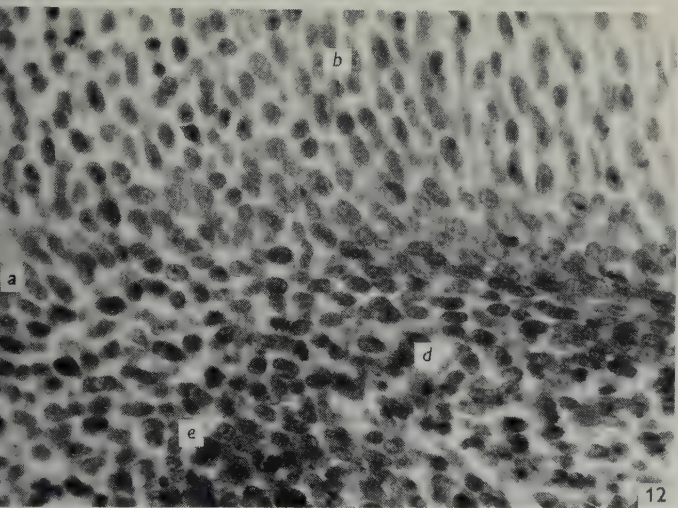
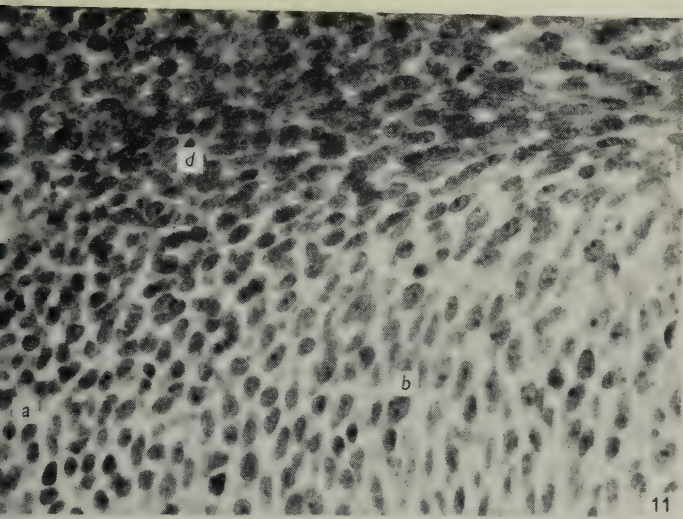
Fig. 13. Posterior part of the perichondrial zone adjacent to epiphysal cartilage (20 days). Note the more loose appearance of the subperichondrial tissue as compared with fig. 12. ($\times 500$.)

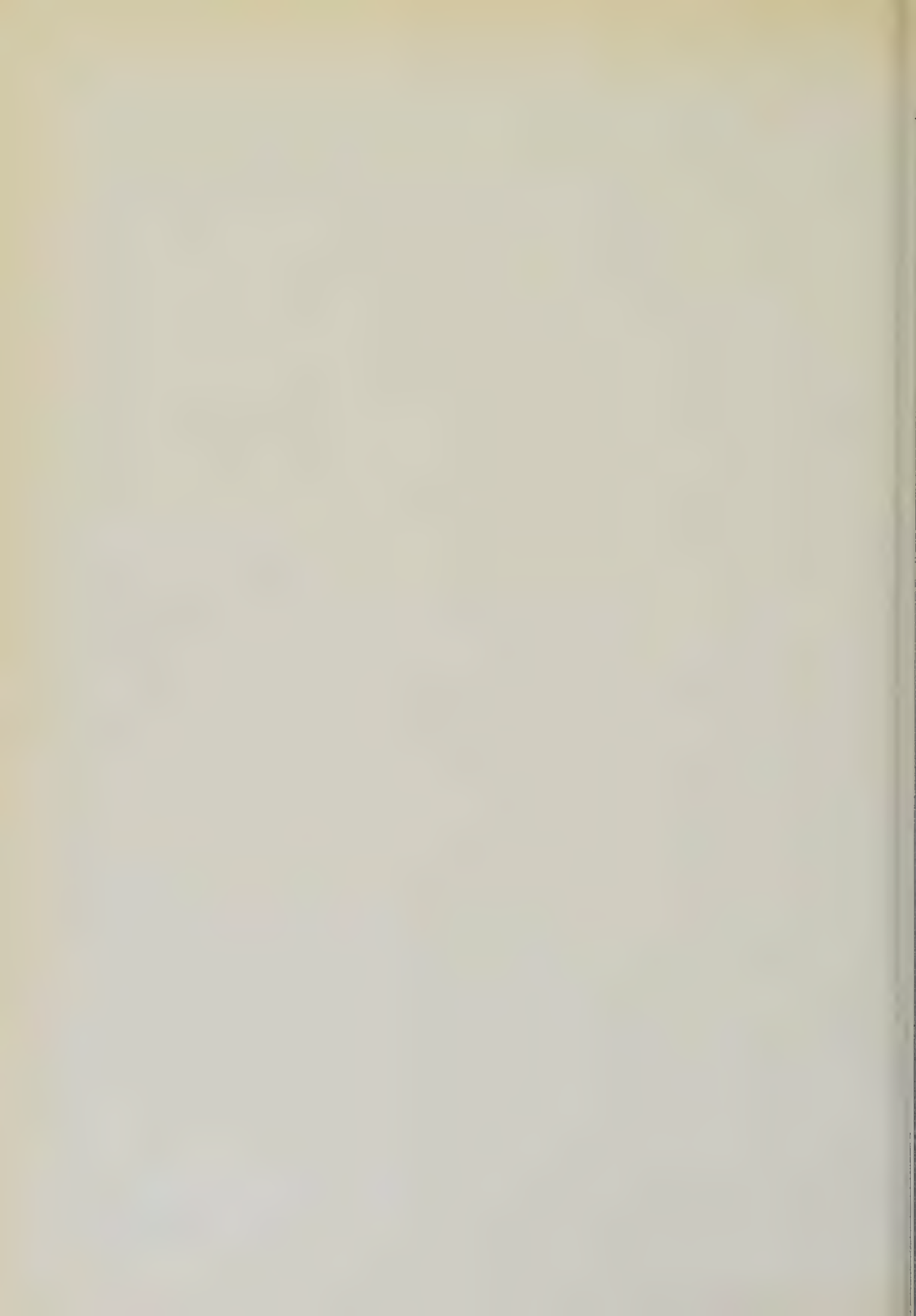
Fig. 14. Anterior part of the perichondrial zone (20 days). Note the laminated appearance of the subperichondrial tissue as compared with the irregular arrangement of cells seen in fig. 13. ($\times 500$.)





PRATT—'PERICHONDRIAL ZONE' IN A DEVELOPING LONG BONE OF THE RAT





EFFECTS OF STARVATION, SEPTICAEMIA AND CHRONIC ILLNESS ON THE GROWTH CARTILAGE PLATE AND METAPHYSIS OF THE IMMATURE RAT

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The effects of a short-lasting period of total starvation, and of pneumococcal septicaemia treated with penicillin, upon the skeletal development of the 25-day-old albino rat have been the subject of a recent experiment (Acheson & Macintyre, 1958; Macintyre, Acheson & Oldham, 1958). Daily records were taken of weight and length of the experimental animals and of their litter-mate controls, and assessments were made of skeletal maturity by radiographing the rats once a week. It was found that the traumatic episode, whether illness or starvation, caused an abrupt slowing of skeletal growth, but that the effect upon skeletal maturation was not so marked. The present paper describes the histological appearances of the tissues in the region of the growth cartilage plate of some of the animals which succumbed during the traumatic episode and of others from a similar experiment carried out more recently.

MATERIAL AND METHOD

Full details of the original experiments have already been published (Acheson & Macintyre, 1958), and a summary of the pertinent facts only will be given here.

Experiment I. Sixty-four albino rats, from eight litters, were used. Thirty-seven of them (seventeen males and twenty females) were inoculated intraperitoneally with a virulent strain of pneumococci at the age of 25 days, and the remaining twenty-seven animals were used as controls. Eighteen hours after inoculation treatment of the infected animals with penicillin was started, but only nine of the experimental group survived. Of the remainder, twenty died within 24 hr. of infection, four died on the third day, three on the fourth day and one on the eleventh day. In the majority of these cases of premature death the control animal was also killed. After death the fore-paw, hind-paw, radius, ulna, humerus, femur and tibia from the right side of the body, and the tail of each animal, were decalcified and sectioned for histological examination. The sections were stained with haematoxylin and eosin.

Experiment II. A study was made of forty rats from five litters. At the age of 27 days, thirty of them (fifteen males and fifteen females) were starved for 48 hr., and then, after 24 hr. *ad lib.* feeding, starved for a further 48 hr., so that they had no food for 4 out of 5 days. Four animals failed to survive the second period of starvation and their bones were decalcified, sectioned and stained in the same manner as was described for Exp. I.

Experiment III. In order to obtain fully calcified bone for sectioning, studies were made of a further group of eleven albino rats, aged 25–30 days, from three

litters; all of these animals were weighed daily for 4 days before they were exposed to illness or starvation to ensure that they were growing normally. Two animals were starved for 48 hr. and killed, together with a freely fed litter-mate control; two were starved for two 48 hr. periods, as described above, and killed with their control. Three were given intraperitoneal injection of virulent pneumococci (type III) but were not treated. Two of these died within 12 hr. of infection and the third was killed after 24 hr. This last group also had a litter-mate control which, as with the other animals, was killed with ether. The head of the humerus, and a few millimetres of the proximal end of the shafts, were dissected from each of the animals. Those from the right side of the body were decalcified, those from the left were sectioned in the fully calcified state. In each case the bone was first cut longitudinally until the sections showed the growth cartilage plate and metaphysis. Then the block was turned, and further sections made at right angles to the long axis of the bone. Some calcified sections were stained with cobalt nitrate, ammonium sulphate and haemalum, and some with haematoxylin and eosin; all the decalcified sections were stained with haematoxylin and eosin.

FINDINGS

The normal growth cartilage plate and metaphysis

The growth cartilage plate is a unipolar structure, that is to say, it grows in one direction only (Ring, 1955). The site of growth is in the reserve layer, where mitosis occurs, and this is situated in immediate relation to the bony epiphysis. As each new cell forms it pushes away its predecessor, thus forming columns of cartilage cells, first of increasing maturity and later of advancing degeneracy. The cells passing through this cycle make up the serial and columnar layers of the growth cartilage plate. The process of degeneration of the cartilage cells has two distinct characteristics: first the nucleus enlarges, disintegrates, and finally disappears, and secondly, the cell itself becomes vacuolated and greatly enlarged (Pl. 1, figs. 1, 4). As a consequence the vessels and osteoblasts of the metaphysis are invading a hollow scaffolding. The uprights of this scaffolding are pressed thin by the vacuolation of the cells between them, but maintain their pliability until their contact with the bone-forming tissue is imminent, when they become calcified lamellae (Pl. 1, fig. 2). The dominant cells at the metaphyseal margin are osteoblasts, which are marshalled in their thousands against the calcified lamellae, where they form bone (Pl. 1, fig. 3).

During rapid growth, which is characteristic of the healthy young animal, calcification does not penetrate far, and much of the cartilaginous matrix between the metaphysis and the reserve layer of the growth cartilage plate is uncalcified (Pl. 1, fig. 2). There is, however, an appreciable distance between the earliest new bone and the osteogenic elements which are most advanced into the cartilage. Capillaries can be traced between the delicate newly calcified lamellae, reaching up as far as the degenerate vacuolated cartilage cells (Pl. 1, fig. 3). Nowhere does this process of invasion appear to be held back or restricted; in fact the osteogenic tissues give the appearance of growing freely into empty spaces created by the degeneration of the cartilage (Pl. 1, fig. 3).

The growth cartilage plate and metaphysis in septicaemia and acute starvation

The changes in the normal pattern which occur in response to septicaemia and to starvation are similar, and will be described together. There is a pronounced decrease in the depth of the growth cartilage plate, which is mostly due to a reduction in the size of the columnar layer (Pl. 2, fig. 5; Pl. 3, fig. 8). Distended and degenerate cells are no longer to be seen at the metaphyseal margin, nor is the delicate intercellular matrix which characterizes normal growth any longer evident. As a consequence, the calcified cartilage, which penetrates as far as the serial layer of the plate, has lost its filigree appearance, and has become stout and thick (Pl. 3, fig. 9); calcification is also visible in many of the septae between the cells of the columnar layer. The effect of this increased penetration of calcification is that whereas in health only degenerate or empty cells are being surrounded by calcification, with slowed growth due to septicaemia or starvation, calcium salts are being laid down in a matrix which has not yet been pressed thin by vacuolation, and cartilage cells which only show the earliest evidence of degeneracy become enmeshed in a calcified network (Pl. 3, figs. 9, 10). In septicaemia these appearances manifest themselves within 24 hr. of the animal showing obvious signs of illness (Pl. 2, fig. 7).

Changes at the chondro-metaphyseal boundary, and in the metaphysis itself, are less dramatic and slower to develop. The line of demarcation between cartilage and newly forming bone is sharper than in health; and the new bone gradually comes nearer to the cartilage, and as this happens the number of osteoblasts becomes reduced (Pl. 2, fig. 6). In contrast the number of osteoclasts and chondroclasts increase, and many of these are to be seen at calcified intercellular septa which seem to act as barriers to free capillary and osteoblastic penetration of the cartilage (Pl. 3, fig. 11). The newly formed bony trabeculae are much thicker than in healthy animals of the same age, and frequently the transverse as well as the longitudinal septae become ossified (Pl. 2, fig. 6).

The growth cartilage plate and metaphysis in chronic illness

One male rat recovered from its initial septicaemia, but a few days later developed an otitis media from which it died aged 36 days, when its litter-mate control, also a male, was sacrificed. Throughout its illness the sick rat was fed on a full laboratory diet which was supplemented with milk given by hand from a dropper. Thus, the considerable interference which took place with its developmental processes cannot be ascribed to starvation in this case.

The growth cartilage plate was very narrow and inactive and a deep blue coloration with haematoxylin suggested extensive calcification (Pl. 4, figs. 12, 13), a suggestion which was supported by the radiographic appearances (Pl. 4, figs. 14, 15). The animal was dead for about 8 hr. before the bones were fixed, so that the changes in cell structure may, in part, be the result of post-mortem degeneration: nevertheless the general acellularity of the metaphysis is unlikely to be entirely due to this cause.

DISCUSSION

Measurements of the animals subjected to starvation or septicaemia had previously shown that growth stopped almost immediately after exposure to these adverse circumstances (Acheson & Macintyre, 1958). Histological studies now indicate that narrowing and increased calcification of the growth cartilage plate accompany the slowing of growth, and that *later* there is a decrease in the rate of osteogenesis in the metaphysis.

The thinning of the cartilage plate suggests that the normal balance between rates of cartilage growth and bone formation is disturbed, and that osteogenesis is, for a time at any rate, outstripping the provision of the cartilaginous scaffolding upon which the new bone is laid down. The altered pattern of calcification whereby calcium salts are deposited deeper and deeper along the interstitial matrix and through the septa of the growth cartilage plate (Pl. 2, fig. 7; Pl. 3, figs. 9, 10) is likewise explicable in terms of slowed cartilage growth and maturation. For whereas in health the distension and vacuolation of the cartilage cells causes the intercellular matrix to be pressed thin before calcification occurs (Pl. 1, figs. 2, 4), in the experimental animals extensive calcification precedes these changes and indeed seems to prevent them from occurring at all.

Osteogenesis continues fairly normally for a while and, as a result, new bone is brought up to the very margin of the cartilage, but then osteoblasts become fewer, and further osteogenesis only proceeds with the help of numerous chondroclasts, which permit capillary penetration by eroding the hardened cartilage.

Finally, however, if the general systemic disturbance continues, the osteoblasts vanish, and the whole process of skeletal development is brought almost to a halt.

These histological appearances in experimental animals are consistent with findings in the living child. Increase in stature is a measure of the chondroplasia in the tibiae, femora and the vertebrae; osteogenesis in the epiphyses can be studied in radiograms where it shows up as a series of shape changes in the shadow of the bony epiphysis (in this context it is usually called 'skeletal maturation') (Acheson, 1954, 1957). Study of these two processes has shown that when a child is sick, or when it lives in a poor home, increase in stature suffers a more serious setback than does skeletal maturation (Acheson & Hewitt, 1954; Hewitt, Westropp & Acheson, 1955; Falkner, 1958). Using similar radiographic methods it has been found that in the rat also longitudinal growth seems much more susceptible to interference than skeletal maturation (Acheson & Macintyre, 1958). Thus, the clinical and histological evidence go to support the suggestion already made by Park and his collaborator Follis (Follis & Park, 1952; Park, 1954) that chondroplasia and osteogenesis are dissociable. The nature and degree of dissociation would seem to depend upon the duration and severity of the adverse experience.

Pathogenesis of lines of increased density in radiographs of growing bones

Although Stettner (1920, 1921) and Harris (1926, 1931) both realized that a line of increased density in the radiogram of the metaphysis indicated that a child had suffered a period of arrested or slowed growth, Follis & Park (1952) were the first to suggest that a dissociation between chondroplasia and osteogenesis was the

immediate cause of such lines. They differentiate between a 'transverse stratum' of thickened bone, and a 'growth retardation lattice' of calcified cartilage, both of which are radio-opaque. The first, they believe, is due to continued osteoblastic activity when cartilage growth has slowed, the second to 'the continued growth of the cartilage' with 'osteoblastic and vascular failure' (Follis & Park, 1952). They state (*loc. cit.*) that 'transverse strata in bones may be the result of illnesses of a most temporary and relatively mild nature', whereas 'lattice formation is the result of a growth disturbance of a number of days or weeks' such as 'the severe pneumonias following whooping cough'. This hard and fast differentiation between the two is almost certainly artificial. The formation of a calcified lattice (the penetration of calcium salts deep into the cartilage) followed immediately upon systemic disturbance in the rats discussed in this paper; Harris (1933) commented upon similar changes in puppies which were starved for 72 hr. It is a little more than an exaggeration of the physiological calcification of cartilage which is an essential step in normal bone formation; and the thickened trabeculae illustrated in Pl. 2, fig. 6, and Pl. 3, fig. 11, are evidently the result of ossification occurring on the bulky cartilaginous matrix of the growth retardation lattice. These thickened trabeculae show up very clearly in the radiogram of the metaphysis as a dense shadow and, in animals which survived the systemic disturbance, radiographs taken after recovery revealed a classical 'line or arrested growth' in the diaphysis. In cases where the systemic disturbance is protracted and osteoblastic activity diminishes, the retardation lattice will have less and less bone formed on it, and eventually will itself become the principal reason for a dense shadow in an X-ray of the metaphysis.

It seems, however, that even in the most unfavourable conditions cartilage growth does not come to a *complete* halt. Study of serial radiograms of children in prolonged coma due to tuberculous meningitis show that a certain amount of new bone is still being formed at the metaphysis (Acheson, 1958, and unpublished data). In the experimental animal, Winters, Smith & Mendel (1927) and Quimby (1951) found that immature rats, whose weight was held constant for several weeks, continued to enlarge their skeletons a little, and Follis & Park (1952) observed some growth occurring in the ribs of chronically ill children, which post-mortem were found to have a pronounced 'growth retardation lattice'.

There is a considerable amount of evidence to suggest that the pars anterior of the pituitary gland undergoes atrophic structural changes during starvation which involve, in particular, the acidophil cells (Jackson, 1917; Meyer, 1917; Sedlezky, 1924; Stefko, 1927; Kylin, 1937) and that in such circumstances, there is some withdrawal of the somatotrophic and other hormones (Kylin, 1937; Werner, 1939; Mulinos & Pomerantz, 1940; Stephens, 1941; Vollmer, 1943). Furthermore, it has been shown that anterior pituitary extract, given as a supplement to normal feeding, after the starvation of young rats, improves the quality of recovery (Quimby, 1951; Fábry & Hrůza, 1956).

It is well known that normal cartilage growth cannot take place without adequate secretion of somatotrophic hormone (Asling, Simpson, Li & Evans, 1950, 1954; Ray, Simpson, Li, Asling & Evans, 1950; Ray, Asling, Walker, Simpson, Li & Evans, 1954; Simpson, Asling & Evans, 1950), so it may be postulated that the slowing of chondroplasia in the starved rat is due to the withdrawal of the somatotrophic

hormone, and that a similar mechanism is brought into action during septicaemia and other illness. The phenomenon may, in fact, be looked upon as an example of what Hubble (1957) has called endocrine homeostasis.

SUMMARY

The changes evoked by acute starvation, pneumococcal septicaemia or chronic otitis media, in the growth cartilage plates and metaphyses of immature rats are described. There appears to be immediate slowing of chondroplasia, with more extensive calcification of the cartilage than is normal, followed later by a reduction of osteoblastic activity. The pathogenesis of lines of arrested growth, often visible in the radiogram of the metaphysis of the growing child, is discussed in the light of these findings. It is suggested that withdrawal of the somatotrophic hormone of the anterior pituitary gland may initiate the changes in starvation, and possibly also during septicaemia and other illness.

I should like to express my gratitude to my colleague, Dr Neil Macintyre, Department of Anatomy, Western Reserve University, Cleveland, Ohio, with whose collaboration the animal experiments were performed. Dr Michael Fry and Dr Joan Mullaney, of Departments of Physiology and Pathology, Trinity College, Dublin, have discussed the problem with me and offered much help and advice. The histological sections are the work of Mrs Eleanor Oldham and Mr Larry Wilmott, and the photomicrographs that of Mr Glen and Mr Murray. Dr Macintyre receives financial support from the National Science Foundation (U.S.), the Committee for Research for Problems in Sex, The U.S. National Academy of Science, and the U.S. National Research Council. Much of this research was carried out during the tenure of the Radcliffe Travelling Fellowship, University College, Oxford, and a Rockefeller Travelling Fellowship in Medicine.

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EXPLANATION OF PLATES

In the majority of the animals several bones were studied, but in any individual the appearances of growth cartilage and metaphysis were, at the age groups under consideration, remarkably consistent. Therefore all the sections illustrated (figs. 1-13) are from a single representative region, the proximal end of the humerus. The radiograms, however (figs. 14, 15), are of the lower limb and tail which, because of their size, are more suitable for illustration by this method than the humerus.

PLATE 1. Appearance in health

Fig. 1. Healthy female 29 days old. Note the depth of the cartilaginous growth plate. (Rat F. 4, H. & E.) $\times 70$.

- Fig. 2. Healthy male, 27 days old. Calcified cartilage, which is black in the photograph, has not penetrated very deeply into the plate. Note that the intercellular matrix has been pressed thin by vacuolation of the cartilage cells before calcification has occurred. (Rat BJ. 6, cobalt sulphide.) $\times 240$.
- Fig. 3. Same section as fig. 1. Note that, despite the intense osteoblastic activity, it is only towards the bottom of the photograph that much bone has been formed upon the calcified lamellae. (H. & E.) $\times 280$.
- Fig. 4. Transverse section from same block of tissue as illustrated in fig. 2. The degeneracy and vacuolation achieved by the cartilage cells before the matrix becomes calcified are again shown. (Cobalt sulphide and haemalum.) $\times 670$.

PLATE 2. Appearance in septicaemia

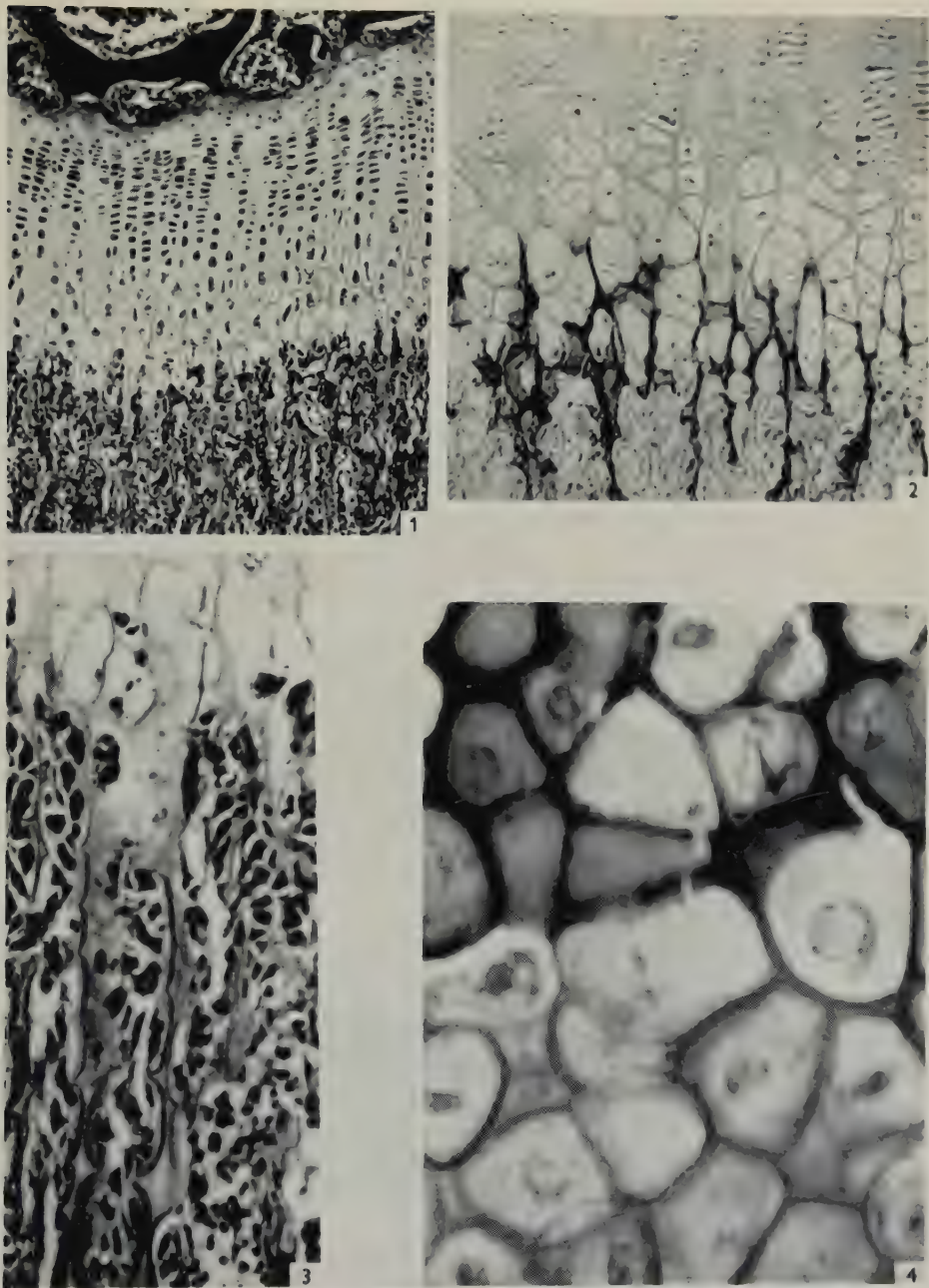
- Fig. 5. Male which died aged 28 days, 3 days after developing septicaemia. The growth cartilage is not so deep as that of the healthy litter-mate shown in fig. 1, and the greater part of this change has occurred in the columnar layer. (Rat F. 2, H. & E.) $\times 70$.
- Fig. 6. Same section as fig. 5. Compare with healthy animal shown in fig. 3 and note bone at margin of cartilage, thickened trabeculae with ossification of transverse septae, and decrease in number of osteoblasts. (H. & E.) $\times 280$.
- Fig. 7. Female aged 30 days, killed 24 hr. after an intraperitoneal injection of pneumococci. Compare with fig. 4 and note that the cartilage cells, which are at an early stage of degeneration, show little, if any, vacuolation; the calcified intercellular matrix is therefore thick. (Rat BK. 8, Cobalt sulphide and haemalum.) $\times 670$.

PLATE 3. Appearance in acute starvation

- Fig. 8. Female aged 30 days, starved 4 days out of 5. Compare with figs. 1 and 5 and note that there is again a pronounced decrease in depth of the growth cartilage. (Rat AJ. 7, H. & E.) $\times 70$.
- Fig. 9. Male aged 30 days, starved 4 days out of 5. In contrast to fig. 2, calcification extends for the entire depth of the columnar layer and reaches the reserve layer. The thickening of the calcified matrix is also seen. (Rat BH. 2, cobalt sulphide.) $\times 280$.
- Fig. 10. Female aged 30 days, starved 4 days out of 5. Thickening of calcified matrix is again shown. The cartilage cells are a little smaller and less degenerate than those in the animal which was only sick for 24 hr. (see fig. 7). (Rat BH. 3, cobalt sulphide and haemalum.) $\times 670$.
- Fig. 11. Section shown in fig. 8. Bony trabeculae are thicker than those in the animal which had septicaemia for 3 days (fig. 6). Osteoclasts, however, seem to have caused erosion at the junction of metaphysis and cartilage, and numerous chondroclasts are also to be seen. There is very little osteoblastic activity. (H. & E.) $\times 280$.

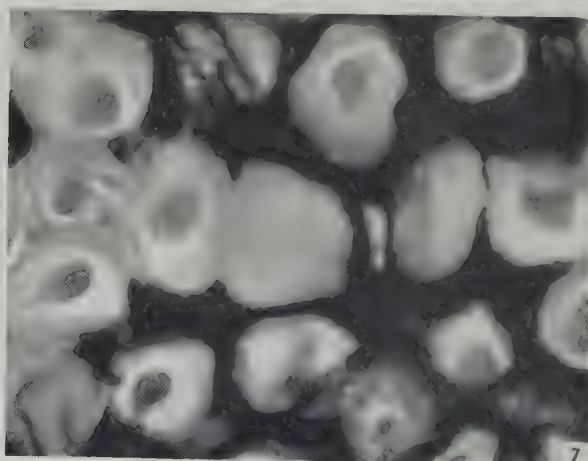
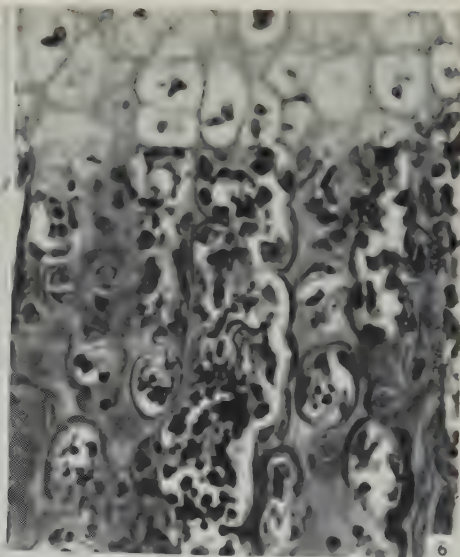
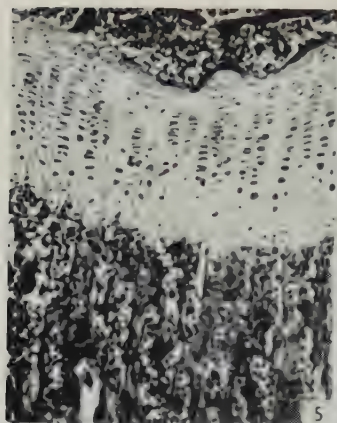
PLATE 4. Appearance in chronic illness

- Fig. 12. Male aged 36 days, which died after suffering from otitis media for 7 days. The growth cartilage, which should be distinguished in this figure from the epiphyseal cartilage, shows considerable narrowing. (Rat K. 1, H. & E.) $\times 70$.
- Fig. 13. Section shown in fig. 12. The metaphysis, the trabeculae of which are greatly thickened, consists of osteoid deposited on calcified cartilage matrix. The cellular changes in this section may be due to post-mortem degeneration. (H. & E.) $\times 280$.
- Figs. 14, 15. The lower limbs and tail, at death, of the animal shown in figs. 12 and 13, compared with those of its litter-mate control (rat K. 3), which was radiographed simultaneously. The points to note in the sick animal (fig. 15) include its smaller size; a dense shadow caused by calcification at the metaphyses of tibia, metatarsals, proximal phalanges, caudal vertebrae, ischia, etc.; narrowing of the growth cartilage plates; general osteoporosis and, at the top left corner, shadows cast by intestinal contents, supportive evidence that the animal continued to eat well till death. Below the proximal metaphysis of the tibia a 'line of arrested growth' is visible, which was formed during the original septicaemia.

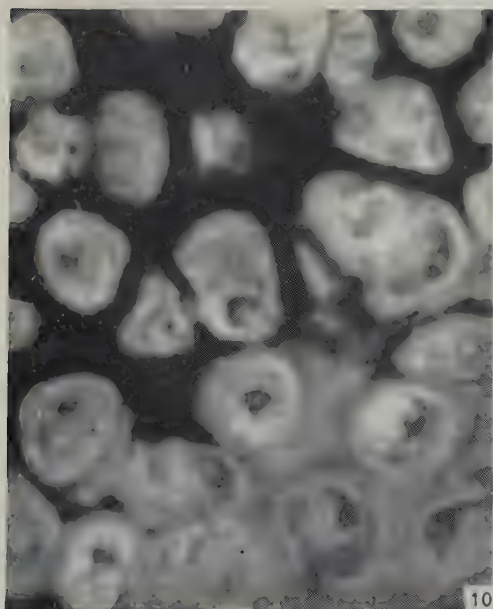
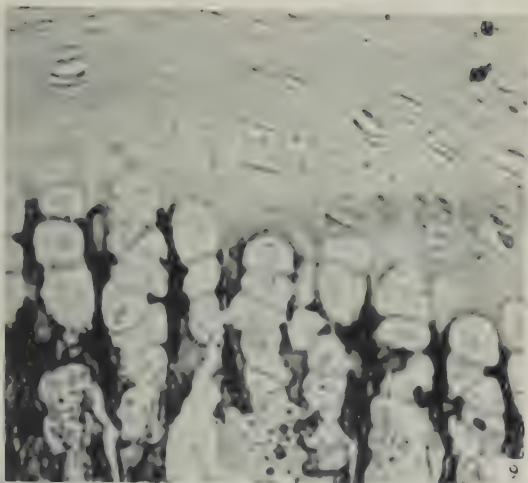
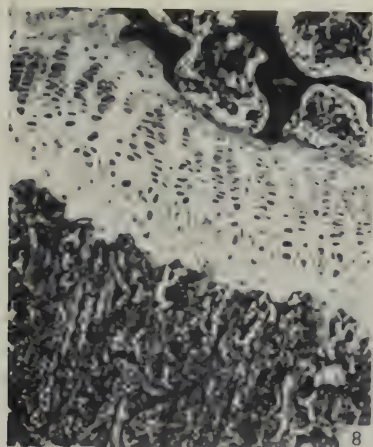


ACHESON—EFFECTS OF STARVATION, SEPTICAEMIA AND CHRONIC ILLNESS

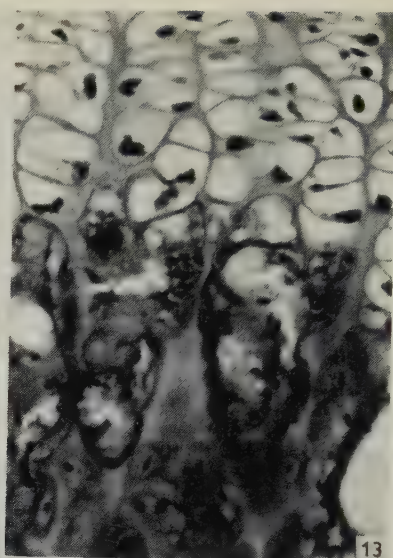
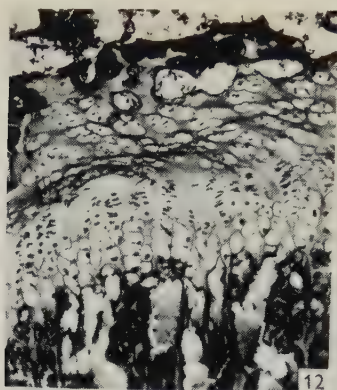
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ACHESON—EFFECTS OF STARVATION, SEPTICAEMIA AND CHRONIC ILLNESS



ACHESON—EFFECTS OF STARVATION, SEPTICAEMIA AND CHRONIC ILLNESS



ACHESON—EFFECTS OF STARVATION, SEPTICAEMIA AND CHRONIC ILLNESS

AN UNUSUAL HUMAN ANTERIOR CEREBRAL ARTERY

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The following unusual features in the branching of the internal carotid artery were seen during the routine dissection of a male cadaver aged 66 (see Fig. 1).

(a) The right internal carotid artery divided into two large branches ('anterior cerebral' and 'middle cerebral') immediately after piercing the dura. The ophthalmic artery on this side was replaced by an anastomosis through the sphenoidal fissure between the anterior division of the middle meningeal artery and the lacrimal artery. The posterior communicating and anterior choroidal arteries almost certainly arose from the branch corresponding to the 'middle cerebral artery' a few millimetres from its origin, but unfortunately it was not possible to check this.

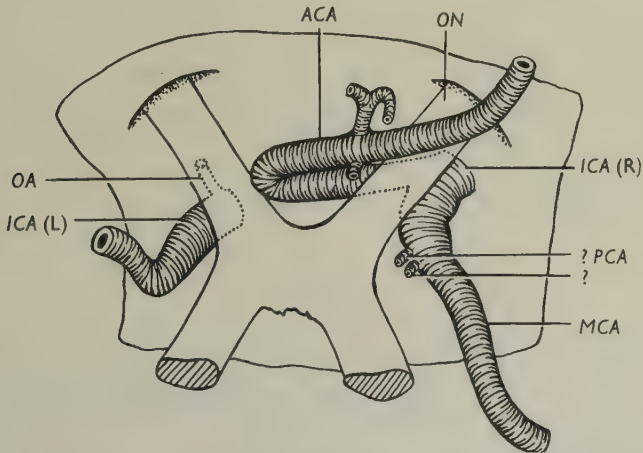


Fig. 1. The superior aspect of the specimen showing the abnormal R. internal carotid artery and its branches. *ACA*, anterior cerebral artery; *ICA*, internal carotid artery; *MCA*, middle cerebral artery; *OA*, ophthalmic artery; *ON*, optic nerve; *PCA*, posterior communicating artery.

(b) The large right anterior cerebral artery passed *inferior* to the right optic nerve emerging between it and the left nerve to reach the longitudinal fissure. It was impossible to decide whether there had been a left anterior cerebral artery because the dissection was at an advanced stage when the anomaly was discovered. There was a normal left ophthalmic artery.

In none of the many accounts of the variations of the cerebral arteries and circle of Willis in man (e.g. Mitterwallner, 1955; Duroux, Dujol, Avet & Gabrielle, 1954; Adachi, 1928; Lautard, 1893) have I found any reference to an anterior cerebral artery like the one described here. So far as I know, no such variation has been reported in the extensive literature on the angiography of cerebral vessels.

The nearest comparable anomalies which have been described in man are inter-carotid anastomoses (Incoronato, 1872; Mitchell, 1889). Among the descriptions of cerebral vessels in other vertebrates, there is one report of an artery similar to the present one, in a chimpanzee (Watts, 1934, fig. 6). It arose, however, from an inter-carotid anastomosis.

Sometimes an arterial anomaly represents the persistence in the definitive state of a vessel temporarily of major importance in the embryo, but which disappears during the course of development. Accounts of the development of the vessels in this region (Padget, 1948; de Vriese, 1907; Mall, 1905; Tandler, 1902) were studied, but no major vessel described could be regarded as a possible precursor for the anomaly.

It therefore seems more likely that the anomalous artery represents the enlargement of a channel normally of small size in the embryo and in the adult. Accounts of the vessels of the chiasmal region were examined, and it seems that the unusual artery may have arisen by enlargement of an anastomosis between the superior hypophyseal branches of the internal carotid artery proximal to its posterior communicating branch, and the anterior cerebral artery (McConnell, 1953, fig. 4; Xuereb, Prichard & Daniel, 1954, fig. 6)—corresponding probably to the vessels encircling the optic nerve described by Godinov (1929) and included by Abbie (1938) in the chiasmal plexus of arteries. The commencement of the true anterior cerebral artery has disappeared. Probably the 'branch' which appears to be the middle cerebral artery is the continuation of the internal carotid artery, for the middle cerebral always arises from the internal carotid distal to the posterior communicating artery. Unfortunately, however, it was not possible to be sure which was the posterior communicating artery in the specimen.

SUMMARY

An anterior cerebral artery is described arising from the right internal carotid immediately after it pierced the dura, and passing inferior to the right optic nerve to emerge between it and the left nerve. The internal carotid artery on this side had no ophthalmic branch. No record could be found of a comparable anomaly in man. The possible origins of this vessel are discussed.

I wish to thank Miss M. Ogilvie for her valuable help in preparing the figure.

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A METHOD FOR STAINING WHOLE BRAINS FOR GROSS AND MACROSCOPIC STUDY

BY W. HEWITT

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A number of staining techniques are available for the macroscopic examination of thick slices of brain. Examples are those of Mulligan (1931) or Tompsett (1955), which stain the grey matter; or those of Waldman & Michaels (1954) or Brody & Wirth (1957), which colour the white matter with sudan stains.

In all of these only the surface of a previously cut slice is stained and surface staining has a number of disadvantages. The techniques of Mulligan (1931) and Tompsett (1955) require a painstaking removal of the membranes and blood vessels, before sections are cut, to prevent smearing of the grey and white matter. Even then success is by no means always assured. Surface-stained brain slices will also be spoilt, by exposure of unstained areas underneath, if accidentally knocked or chipped. Moreover, the section originally made may not be the one desired and it is not possible to pare away the surface to the desired level.

Apart from these disadvantages, it can be useful to stain a whole brain so that when it is sectioned the grey and white matter can be clearly distinguished. For these reasons a technique has been developed for staining a whole brain using sudan stains. This was devised before the writer was aware of the staining methods of Waldman & Michaels (1954) and Brody & Wirth (1957); it is in fact based on a different principle.

METHOD

The brains can be fixed in either 70 % alcohol or, for preference, 10 % formaldehyde. The membranes and blood vessels need not be removed beforehand.

The staining fluid used consists of a saturated solution of Sudan III and Sudan IV in a mixture of equal volumes of 70 % alcohol and acetone (prepared by adding a mixture of 0.5 g. of Sudan III and 0.5 g. of Sudan IV to every 100 ml. of alcohol acetone mixture). The brain is immersed in ten times its own volume of this fluid. A smaller volume than this can, however, be used provided it is resaturated whenever the fluid becomes noticeably paler and clearer in colour.

When staining is complete the brain will be a uniform red colour. The time required for this is difficult to judge and is governed by the size of the brain. A cat's brain requires at least a month; a human brain not less than 6 months. The completeness can be assessed by cutting through the brain stem about 5 mm. from its lower end, and if staining is complete at this level it may safely be assumed to be complete elsewhere.

When staining is completed, differentiation is commenced by immersing the brain in 70 % alcohol. This fluid is changed at intervals when it becomes deep red in colour, and a quantity ten times the volume of the brain at first requires changing at weekly intervals, but later the time between changes lengthens. Differentiation

is complete when the alcohol only slowly changes to an orange red colour and does not deepen in colour beyond this. In general the period of differentiation equals the time required for staining.

The stained brain can be stored in 70 % alcohol or 10 % formaldehyde, but once it has been dissected or sliced it must be stored in formaldehyde. If a cut surface is exposed to alcohol it becomes pale in colour. When the cut brain is stored in formaldehyde the fluid slowly becomes tinged pink. This is probably derived from stain present in the membranes, ventricles, choroid plexuses or the substance of the brain. After several changes, the formaldehyde usually remains clear.

DISCUSSION

This technique has been applied successfully to several animal brains as well as a human brain. When stained and differentiated the white matter is red in colour and can be clearly distinguished from the grey matter, stained a pale pink (Pl. 1).

The principle of the technique depends upon the affinity of sudan stains for lipids and the myelin of the white matter is thereby coloured. The staining is presumably effected because the alcohol acetone mixture is used as a solvent for the stain and alcohol alone is used for differentiation. The alcohol acetone solution of the sudan gives a higher concentration of the stain than when the solvent used is 70 % alcohol in which sudan is less soluble. On the other hand, the lower solubility of sudan in 70 % alcohol makes the latter a suitable agent for differentiation and the sudan is selectively removed from the brain tissue other than the myelin for which it has a greater affinity.

Very effective results are obtained with this technique when sections of the brain are made. These are no trouble to prepare and may be of any thickness from 15μ upwards. The thinner sections can be cut on a freezing microtome from thicker slices of the brain (about 1–2 cm. thick) which have been previously washed for 24 hr. These are much enhanced by counterstaining with haematoxylin. Ehrlich's acid haematoxylin, differentiated with acid alcohol, has been successfully used for this and the sections mounted in glycerine jelly. Thicker sections can be made with a knife and stored in the manner already described.

This method could prove a useful aid to dissecting a brain if a successful counterstain for the grey matter could be devised. A number of experiments with this aim in view have been carried out but none have given entirely satisfactory results. Very dilute methylene blue is an adequate counterstain but washes out when stored in aqueous solutions, and even the use of the alcohol soluble dye has failed to prevent this. Bouin's fluid, which colours the grey matter yellow, has the same defect unless the brains are stored permanently in this fluid. Up to the present, the most successful counterstain has been a 0.1 % solution of Methasol Fast Yellow R.S. (Imperial Chemical Industries Ltd.) in 70 % alcohol. This penetrates a whole brain imparting an orange yellow colour to the grey matter and tinging the red of the white matter a faint orange. There is a slight tendency for the dye to pass into solution when the brain is first transferred to formaldehyde but after this the colour remains fixed. The disadvantage of all the counterstains used has been that in addition to the cell bodies the axons are also stained and the red colour of the myelin becomes affected.

SUMMARY

A method is described whereby the white matter of whole brains is stained red with Sudan III and Sudan IV. The use of counterstains for the grey matter is discussed.

I am indebted to the Dyestuffs Division of Imperial Chemical Industries Ltd. for their help by suggesting the use of Methasol Fast Yellow R.S. as a counterstain. My thanks are also due to Mr G. A. Langridge, of the Photographic Department of St Thomas's Hospital Medical School, for technical assistance.

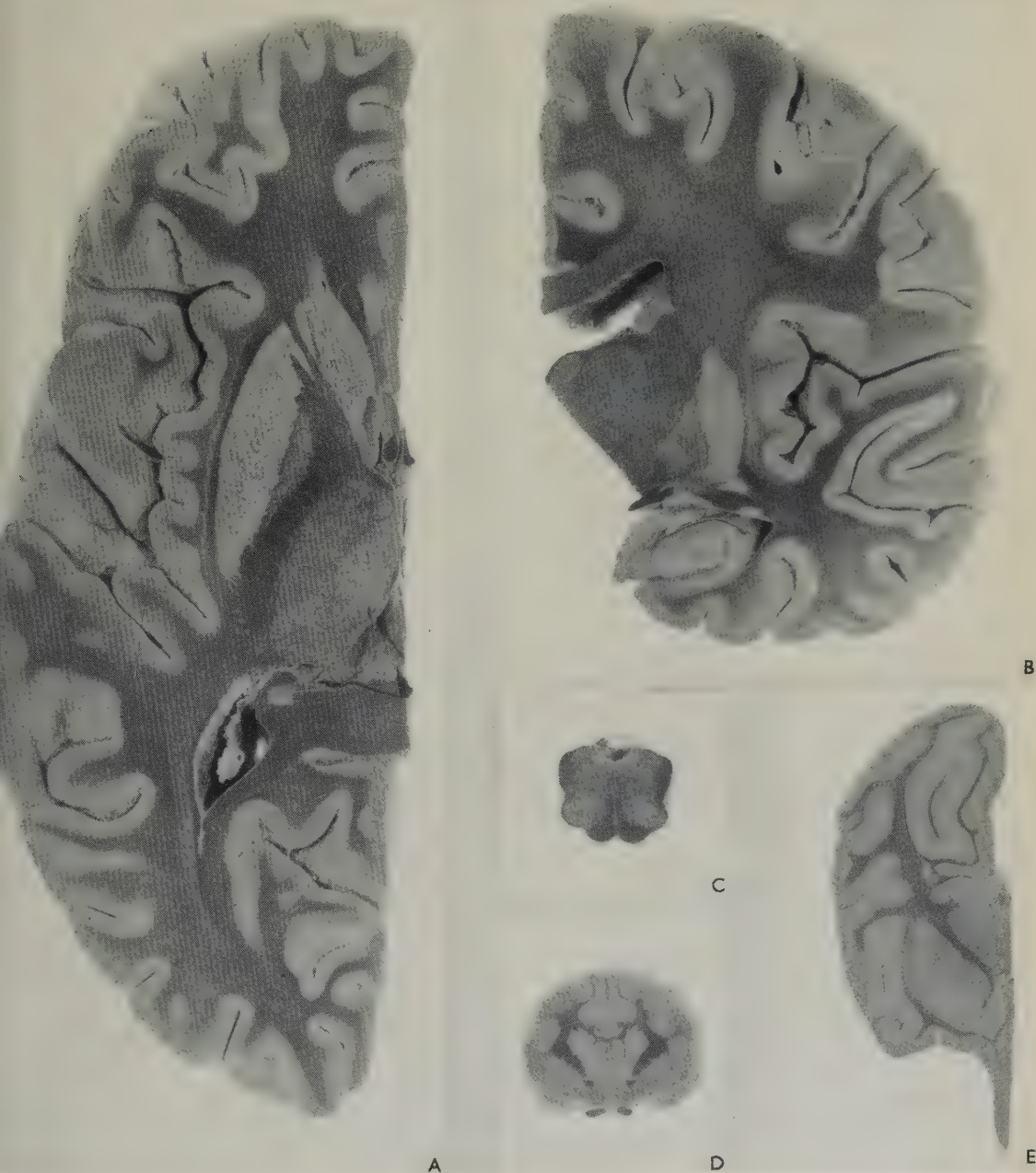
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EXPLANATION OF PLATE

Thick slices made through various brains previously stained with Sudan III and Sudan IV mixture but not counterstained. All are actual size.

- A. Horizontal section through human cerebral hemisphere.
- B. Coronal section through human cerebral hemisphere.
- C. Transverse section through human medulla oblongata at the level of the olivary nuclei.
- D. Coronal section through the brain of a domestic cat.
- E. Horizontal section through cerebral hemisphere of a macaque monkey.



HEWITT—STAINING WHOLE BRAINS FOR GROSS AND MACROSCOPIC STUDY

(Facing p. 136)

REVIEWS

Chemical Anthropology: a New Approach to Growth in Children. By ICIE G. MACY and HARRIET J. KELLY. (Pp. 149; 28s.) Chicago University Press. 1957.

In 1923 a Nutrition Research Laboratory was set up at the Merrill-Palmer School in Detroit, an institution started a few years previously for the purpose of training young women in the study of child development and in the practice of rearing children according to the best scientific opinion. The Merrill-Palmer School continues to flourish, and contributes from time to time data of importance in the child development field. Dr Icie Macy and Dr Harriet Kelly are both consultants there, the first in nutrition, the second in statistics. When in 1931 the Nutrition Laboratory moved to the Children's Fund of Michigan Dr Macy, its director, made a particularly intensive and detailed metabolic study of a very small number of healthy children. Chemical balance studies of nitrogen, phosphorus, sodium, chloride and other substances were made initially for 55 days on eleven children; then later for 225 days on another eleven children and 4 years after this for 55 days on seven of the second eleven. Some most interesting material on the metabolism of children in the 4- to 12-year age range was thus accumulated. Dr Macy published the results of this experiment in 1942 in *Nutrition and Chemical Growth in Children*, Vol. 1, adding the original raw data in Vols. II and III in 1946 and 1951 respectively.

Now we have a fourth volume ostensibly based on the same data, but in fact going beyond them and travelling, it seems to the reviewer, in a somewhat indeterminate direction. The tables of data in this book present solely mean values of an unspecified number of children divided into three age-groups 4-6, 7-9 and 10-12. They are thus clearly illustrative only, and in no sense to be regarded as evidence for the statements made in the text. The research worker active in the field will find nothing to interest him here; the first three volumes are the ones for him.

The text seems designed rather for the undergraduate student of child development or domestic science. It discusses in an elementary and clear, though not very concise, fashion 'basic concepts of growth'; the absorption and net retention of calcium, magnesium, sodium, potassium, phosphorus, chlorine, sulphur and nitrogen; body composition; and the changes of these last items with age between 4 and 12. Much of this is very interesting. There are data, for example, on gastric emptying time after meals of water, milk, carbohydrate, cream and meat (each child given a barium meal for each test, with goodness knows how much total radiation delivered, though the authors were amongst a very large company of sinners at the time they did the experiments). There are data on the number of bowel movements per 24 hr. (1.8 in 4-6 group, 1.6 in 7-9 and 1.3 in 10-12); on the weight of faeces as the percentage of fresh food intake (approx. 5%); on the faecal content of cellulose and of the various minerals. Both the absolute amounts of minerals retained per day by children of this age and the percentage of the daily intake retained are given; for example, some 6-13 m-equiv. of calcium are retained per day, which is around 20% of the daily intake.

Through such information a picture is built up of the child laying down new tissue and changing gradually his percentage of fat, muscle and bone. Naturally the study is too restricted in numbers to give more than very general trends, but it does serve to point the way towards an analysis of human growth in chemical terms. It is this that the authors term chemical anthropology. Dr Macy and Dr Kelly are pioneers in the subject, and their careful, desperately painstaking spade-work deserves the warmest recognition. Perhaps this book does it less than justice. Certainly the publisher's blurb, one of the worst the reviewer has yet come across, claims far too much and hence produces disappointment. But behind this rather patchy, rather thin façade lie some well-dug, solid foundations. If this book sends some readers back to the 1942 volume it will have served a useful purpose, and if it stimulates, as it certainly will, the interest of child-development students in biochemical matters, it will more than justify the labour of its writing. J. M. TANNER

Morphological Integration. By EVERETT O. OLSON and ROBERT L. MILLER. (Pp. xv + 317; 75s.) Chicago University Press (Cambridge University Press). 1958.

This book is an application of the techniques of multivariate analysis to the profoundly important problem of the assay of form. Its authors are palaeontologists and are therefore preoccupied with bones; their techniques are applicable in principle to soft parts—which is not to say that such an application would be feasible in practice. Although their book is long—far too long, many will think—their conception of the problems that might reasonably be held to fall within the subject matter suggested by its title is rather narrow. This is most clearly shown by the fact that the names of Ashton, Zuckerman and Bronowski are not mentioned. Reference to the work of Kermack (misspelled) is confined to a single footnote, and D'Arcy Thompson is not thought worth mentioning at all.

In bare outline, Olson and Miller proceed thus. The shape of an animal, or of part of an animal, is expressed as an assemblage of linear measurements. For example, the form of the pigeon's skeleton is given in terms of twenty-six measurements—height of occiput, length of femur, depth of keel, width of metatarsal complex, and so on. The measurements are repeated upon a large number of specimens (of pigeons, a hundred), whereupon it becomes possible, by applying well-known statistical methods, to find out which measurements are intercorrelated and which vary independently. If an unexact criterion of correlation is taken, the number of correlations established by it is unworkably large; but it may be reduced by raising the entrance qualification, i.e. by raising the value of the correlation coefficient below which no pairs of correlations are admitted. The measurements can now be seen to fall into groups of two upwards, 'primary ρ -groups', defined by the property that each measurement in the group is correlated with every other. It will often happen that a given measurement falls into more than one such group, so that the ρ -groups overlap and intersect; and this makes for difficulties in analysis. The next stage, then, is progressively to raise the acceptable value of the correlation coefficient; the less closely correlated pairs of measurements fall away, and one may eventually be left with a small number of 'basic pairs' of correlations, defined by the property that each member of the basic pair is more highly correlated with its partner than with any other measurement. The basic pairs are the nuclei around which non-overlapping sets of ρ -groups can be formed: all ρ -groups that do not contain both terms of at least one basic pair are dropped, and from these reduced groups are eliminated all those single measurements of basic pairs whose partners do not occur within them. The formal connexions embodied within the final, 'basic-pair ρ -groups' are thought to disclose or reflect real biological connexions, and evidence is given that they do so.

The authors now attempt to give an exact meaning to the idea of 'morphological integration', i.e. to the idea which we think of rather vaguely as standing for the knitted-togetherness of individual parts and the degree of complexity of their mutual arrangements. 'Bonds' are the correlations (of some chosen degree of strength) between pairs of measurements. The index of morphological integration is the product of two quantities, roughly—for the rigorous definition is lengthy—(a) the ratio of the number of recorded bonds to the number of completely intercorrelated ρ -groups in the system under investigation; and (b) the ratio of the number of observed bonds to the number of possible bonds in the system. The two terms in the product are intended to give acceptable meanings to two essential ingredients of the idea of integration, viz. the pervasiveness and the strength of the connexions between parts.

The results of applying this concept are, so far, meagre and unsatisfying. It turns out, for example, that there are strange ups and downs in the degree of morphological integration of the skeleton of the rat as it develops, and, over secular periods, in certain lineages as they evolve. Biological interpretations are generally inexpert; indeed, no such interpretation can be arrived at without an exhaustive analysis and classification of the causes of the differences between the measurements taken on one fossil and another. The authors would benefit greatly from, for example, a careful study of Waddington's *The Strategy of the Genes*. It must also be said that, in spite of the space available to them to deploy their

thought, the authors have not got the knack of clear exposition, and their style is ponderous. Yet, in spite of these shortcomings, the authors have the courage of their convictions; they have really *done* something and worked hard at a most difficult and (it must sometimes have seemed to them) unrewarding problem. We must not base a final opinion of the value of the enterprise itself upon a book that, like D'Arcy Thompson's, does not claim to be much more than preface.

P. B. MEDAWAR

Human Dissection: its Drama and Struggle. By A. M. LASSEK. (Pp. 320; 17 illustrations; £2. 10s.) Oxford: Blackwell Scientific Publications. 1958.

Many of the most dramatic incidents in the history of medicine are concerned with anatomy. The struggles of anatomists against superstition, primitive beliefs, taboos, religious bigotry and other handicaps are clearly revealed in this book which is, especially in its first half, essentially a brief history of anatomy.

The second half of the book gives information about the development of dissection in Europe, Asia and North America during the last three or four centuries and is very uneven in content. Some countries are not mentioned, about nine pages are devoted to continental European countries and Asia, but about 130 pages are allocated to descriptions of the development of human anatomical dissections in Britain and the United States of America.

One cannot comment on the accuracy of much of the information given about other countries, but the details about practice and procedure in this country are not always correct; and the impression is conveyed that the study of anatomy was confined almost entirely to London and Edinburgh. Other places are mentioned in passing, such as the inaccurate statement (p. 150) that anatomical schools in Sheffield, Bristol, Liverpool and Manchester were closed in 1829 due to lack of bodies. Actually the supplies of 'resurrected' material were so good in places like Manchester and Liverpool that bodies were exported to other centres such as London and Edinburgh. The statement on p. 116 that 'the sole legal supply of bodies by statute in Great Britain until the early part of the nineteenth century, was those of executed felons' is also erroneous. On p. 135 he cites the well-known Shakespearean epitaph against 'body-snatchers' without apparently realizing that it is actually carved on the memorial stone in Stratford-on-Avon.

Despite these and other minor blemishes this book provides excellent reading and can be commended to all anatomists and indeed to anyone interested in the history of medicine.

G. A. G. MITCHELL

Morphologie und Physiologie des Nervensystems. By PAUL GLEES. (Pp. xi + 445; 149 illustrations; D.M. 58.) Stuttgart: Georg Thieme Verlag.

Dr Glees's declared object in writing this book is to give an account of the methods and results of experimental neurology, and especially of the great advances of the last ten years. There are separate chapters on methods, the biochemistry of the nervous system, the receptors, nerve conduction, and the synapse; followed by thirteen chapters on the regional physiology and anatomy of the C.N.S. The treatment is catholic and lively and nothing new or recent, from Freud to ultra-sound, has been forgotten. There are miscellaneous dippings from the literature of many countries and subjects, and the text is studded with references; the lists include 1063 titles, of which some in every chapter, and 89 (or nearly 8½ %) in all, are of Dr Glees's own publications.

In the regional chapters the chief emphasis has been upon function; it was the author's intention that the account of the normal anatomy of the nervous system should be 'limited to the most important facts'. This emphasis is striking, for instance, in the chapter on 'The Reticular System of the Brain-stem'. Perhaps *Morphologie* should not have been so prominent in the title.

The preclinical student would find this a difficult book: the 'neurophysiological and neuroanatomical instruction of the student' is not made easier by the inclusion, without

definition, of difficult terms from psychiatry. If the post-graduate student is aimed at, he should be warned more often and more insistently that the results of animal experimentation may be applied only with caution to the elucidation of clinical problems. This book attempts too much in 420 pages to serve as an advanced text in any one branch of neurology; and for the student at B.Sc. level it is unsuitable because it is sometimes uncritical in acceptance of new results and, while seeming to give a complete and comprehensive account of each topic, it omits key references.

For whom then is this book suitable? Probably for the advanced clinician, to acquaint him with the newer ideas of the experimentalists, and certainly for the more senior English-speaking neurologist, clinical or academic, who wishes to improve his knowledge of the German language and 'literature'—for in general the exposition is clear, the German eminently readable, and the type and layout most attractive.

G. H. WRIGHT

Anatomist at Large. By GEORGE W. CORNER. (215 pp.; \$4.00.) Basic Books Inc. New York. 1958.

Those who have chosen to make their career in the basic medical sciences will be very grateful to Dr Corner for writing his autobiography, for it illustrates how exciting and rewarding an academic life can be in the field of experimental anatomy.

It is clear, particularly to those who know him, that Dr Corner had this thought in mind when he wrote his book, for almost every page illustrates how much he delights in the pursuit of knowledge and would like others to share his enthusiasm. His investigations have helped to satisfy his almost insatiable curiosity and at the same time they have given him the satisfaction of feeling that eventually some of his discoveries may be of value in the practice of medicine. It is this feeling of intellectual satisfaction coupled with a feeling that his work has not been inspired by purely selfish motives which he wishes to convey to his readers, because by so doing he may tempt them to follow in his footsteps. Dr Corner shows himself in this book to be both an eminent scientist and a shy, lovable and generous man, always taking more pleasure in giving than in receiving; thus his laboratory has always been a cheerful and happy place to work in. Dr Corner makes it quite clear that he owes much, if not all, of his happiness and success to his wife who has been a partner to him throughout his career.

He has received many honours but it is apparent that his modesty and integrity always leave him slightly puzzled as to why he should be singled out in this way. In fact he once said to me: 'It is difficult to understand why I should be rewarded for doing what I like.'

Some readers may have wished that Dr Corner had amplified the autobiographical section of his book for it is engagingly written and leaves one wishing to know more about the life of this delightful character. Those who know him, however, are grateful that he was persuaded to write about himself at all; it is certain that he only did so in the hope of persuading a few young men that a career in experimental anatomy can be deeply satisfying and even full of excitement and adventure.

G. WEDDELL

Microscopic Anatomy of the Temporal Bone. By D. WOLFF, R. J. BELLUCCI and A. A. EGGSTON. (Pp. viii+405 and index; 199 illustrations; £5.) London: Baillière, Tindall and Cox Ltd.

This book is essentially a photographic atlas of serial sections of the human temporal bone, adult and infant, cut in the three planes commonly used for study, i.e. horizontal, vertical at right angles to the long axis of the petrous, and vertical in the plane of the long axis of the petrous. In each photograph the main features are labelled, and in amplification there is a very adequate description in the facing text. The arrangement is well planned and very convenient in use.

Otologists of course will find this book of great value, particularly with the advent in recent years of the fenestration operation, but equally there can be few anatomists who

will not gain from it a much fuller appreciation of the anatomy of this highly complicated region. The time and labour involved in preparation must have been enormous and the result is a work which bears the stamp of authenticity.

The quality of the photographs is a little uneven. For the most part they are very satisfactory, but a few are lacking in sharpness and might be replaced in future editions. It would be of value, too, were an Appendix to be included with such information as the serial numbers of the sections illustrated, the magnification of the photographs, and the method of histological preparation. As a help in orientation inset diagrams of the temporal bone, with the middle and internal ears outlined in position, could with profit be appended to the first of each series of illustrations.

There can be no question that this book will prove of real value to all those whose business it is to know the detailed anatomy of the temporal bone—anatomists and otologists certainly, but many physiologists and physicists as well. Its rather high price is a measure of its wealth of illustrations, and for these days may be considered reasonable.

E. W. WALLS

An Atlas of Fetal and Neonatal Histology. By MARIE A. VALDÉS-DAPENA. (Pp. 200; 90s.) London: Pitman Medical Publishing Co. Ltd.; Philadelphia: Lippincott. 1958.

The aim of the author of this atlas is to illustrate the histological appearances of the major organs of the foetus beginning at a stage of about 200 g. (approximately 4 months gestation) ending with a child of $3\frac{1}{2}$ years of age. Because of the fact that changes are taking place rapidly from month to month and the pattern of organs are being converted from a primitive to a complex arrangement, it is essential to have clear concept of the appearance of normal organs. As the author rightly points out, the pathologist working with material from autopsies of foetuses and newborn infants has, up to the present, had no normal histological picture with which to compare the pathological material.

It is readily appreciated from the study of the microphotographs and the brief descriptions which accompany them that organs vary in their responses to changes from an intra-uterine to an extra-uterine environment. For example, the kidney in the premature infant continues to produce new generations of nephrons after birth whereas the foetal zone of the adrenal cease to grow after birth irrespective of whether this occurs prematurely or at full term.

This book will fill a real need for information which is lacking in most text-books of embryology.

W. J. HAMILTON

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- Surface and Radiological Anatomy.* By W. J. HAMILTON and G. SIMON. 4th edition, 1958. (Pp. v+355. 50s.) Cambridge: W. Heffer and Sons, Ltd.
- Evolution of the Speech Apparatus.* By E. LLOYD DuBRUL. 1958. (Pp. vii+103. 36s.) Oxford: Blackwell Scientific Publications, Ltd.
- The Utero-Vesical Junction.* By JOHN A. HUTCH. 1958. (Pp. vii+178. 56s. 6d.) Berkeley and Los Angeles: University of California Press (Cambridge University Press).
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A COMPARATIVE STUDY OF THE NEURONAL PACKING DENSITY IN THE CEREBRAL CORTEX

By D. A. SHOLL

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The differences that can be seen microscopically between stained preparations of various parts of the mammalian cerebral cortex have been studied for over a hundred years. Many attempts have been made to assess these differences and to separate those considered to be biologically insignificant from those thought to be essential to cortical organization. Far reaching generalizations have been built on the qualitative study of Nissl stained sections in which only the perikarya of the neurons are stained. These generalizations were severely criticized in an important paper by Bok (1929) and generally condemned by Lashley & Clark (1946).

The present work is a quantitative investigation of samples made under standard conditions from different well-defined anatomical regions of the cerebral cortex in several mammals. Replicated counts of the perikarya in the same section and in different sections of the same and other brains have been made.

It has been possible to measure the variations in neuronal density between counts on the same brain and between counts on different brains, and to look for an underlying pattern. An attempt has also been made to find a quantitative measure of the degree of similarity between the various cortical regions that have been studied.

MATERIALS AND METHODS

Cat and mouse brains were fixed by perfusing the anaesthetized animals with 10 % neutral formol saline (40 % neutral formaldehyde solution 10 ml., normal saline 90 ml.) after a preliminary perfusion with normal saline. The neutral formaldehyde solution was prepared by shaking the commercial solution with magnesium carbonate and allowing the mixture to stand for a few days. The brains were removed and kept in large volumes of the fixative which was changed whenever a yellowish tinge was shown by the bromo-thymol blue used as an indicator. One human brain was from a 50-year-old man who died from coronary thrombosis; this was perfused through both internal carotids 2 hr. after death. The details of a second human brain were taken from a careful study made by van Alphen (1945); this brain was also from an adult male of unstated age but was fixed in a formol-alcohol mixture.

Blocks about 3 mm. thick were cut from suitable parts of the cortex, dehydrated, cleared in toluene, infiltrated overnight at 37° C. in a mixture of paraffin wax (m.p. 52° C.) with sufficient benzole to remain just molten in a 37° C. oven. The blocks were then transferred to clean wax and finally embedded in 52° C. wax. Sections were cut with thicknesses varying between 10 and 20 μ and stained with 0.5 % aqueous cresyl violet.

Portions of these sections where the pial surface appeared to be plane were selected for examination. The thickness of each section was measured by means of

a calibrated fine adjustment and an oil-immersion objective, focusing first on the under surface and then on the upper surface of the section. These measurements were made at six randomly chosen spots on each section and the mean value of the six differences was assumed to be the thickness of the section under study. Little variation was found between the six values for any section when they were measured in this way but they sometimes differed by as much as $2\ \mu$ from the microtome setting. The depth of the cortex (pial surface to grey/white boundary) of the selected portion of the section was measured with an eyepiece micrometer that had been calibrated against a stage micrometer.

The number of perikarya in a unit volume of cortex may be computed from counts of nucleoli or from counts of perikarya and pieces of perikarya with the application of the Abercrombie (1946) correction. Either method leads to substantially the same result. The counts were made using an eyepiece graticule that enables the image of the section to be divided into a number of strips of known width, length and thickness, each strip being parallel to the pial surface. It is convenient to make the base of the outermost neuron-free layer the outer boundary of the outermost strip. The packing densities (number of perikarya/unit volume of cortex) can then be computed. In the present study the unit volume of cortex was taken as $0.001\ \text{mm}^3$ ($10^6\ \mu^3$).

It is, however, very difficult to make comparisons between the packing densities found for different samples of cortex since the thickness of the cortex, even under standard conditions with a plane pial surface, varies from region to region in the same brain and consequently the total number of strips surveyed may vary from sample to sample. In order to overcome this complication each sample of cortex, excluding the outermost, neuron-free, zone, has been divided into ten strips and the neuronal density computed from the raw data for each of these strips. This figure will be a measure of the neuronal packing density at the 'relative depth' of the midline of this strip. An example will make this process clear. Imagine that the packing density at different depths of a sample has been found by enumerating the nucleoli in twelve strips of cortex each $200\ \mu$ thick. The total thickness of cortex, excluding the outermost layer, is $2400\ \mu$ and we wish to find the mean density in each of ten strips $240\ \mu$ wide. Then the density at relative depth 0.1 will be the mean density of a strip centred at $120\ \mu$, that at relative depth 0.2 will be the mean density of a strip centred at $240\ \mu$ and so forth. These new densities are easily obtained from those found for the observed $200\ \mu$ strips by a simple weighting of observations taken from adjoining strips. No corrections for shrinking, which is presumably of the order 20–25 %, have been made.

Details of the samples used in this study are given in Table 1. These figures do not include the repeated counts that were made on each sample. The absolute figures obtained for such repeated counts did not differ from each other by more than an occasional difference of the order of 2 units in a count of 50. The regions chosen from the cortex are recognizable on anatomical grounds and the reasons for selection are discussed on pp. 154–5.

In this way a number of replicated observations on the neuronal packing density in corresponding parts of the cortex of different adult animals were available for study. A method was then required for making a quantitative assessment of the

total difference with respect to neuronal packing at varying depths between these different regions in the various animals. From a consideration of the means and correlation coefficients subsisting between these variables, statistics known as the Generalized Distances (Mahalanobis, 1936) were calculated. Details of the computations may be found in Rao (1952). The characteristics of this statistical function and its biological significance will be considered later.

Table 1. *Details of sampling*

Animal and cortical region	Numbers of brains studied	Numbers of samples counted	
Man (pre-central gyrus)	2	13	Data for 1 brain from van Aphen
Man (striate area)	2	16	
Man (parastriate area)	2	6	
Cat (anterior sigmoid gyrus)	2	7	
Cat (post-lateral gyrus)	2	10	
Mouse (visual area)	2	5	Data from Haddara

RESULTS

Fig. 1 is a plot of points each representing the neuronal density found at various cortical depths in different sections of the visual cortex from two human brains. The densities are shown with black and open circles to distinguish the observations made from the two brains. This diagram is typical of the wide variation that is found whenever the counts are replicated. It will be noticed that the densities at any given depth show a considerable overlap between the two brains and from any single set of counts it would be impossible to distinguish between the two brains. Various methods of analysing these data are available, and results of using some of these methods will be described.

The total number of neurons in a cylinder of cortex

One may suspect that part of the variation in density may be due to the somewhat arbitrary divisions of the cortex which have to be made in order to study the possible changes in density with depth. If this were a major factor then the total number of neurons contained in a column of cortex under a pial surface of fixed area would be similar in various parts of the same cortical area. Table 2 gives the results of an investigation of this kind for the numbers of neurons in a cortical cylinder bounded by a pial surface with an area of $400 \mu^2$. It will be noticed that the range of the observations, rather than the standard deviation or confidence limits, has been given in the next column. This is because the distribution of the total number of neurons in such a column for any one cortical region is often so far from normality that confidence limits calculated in the usual way would be most misleading. Table 2 shows that not only do the mean number of neurons in these cortical columns for different cortical areas differ, but that, for the samples of human cortex considered, their ranges do not overlap. It may be noted that columns in the areas of cat cortex studied that are presumably comparable in function with areas of human cortex have neuronal populations of approximately the same sizes and within similar ranges. The neurons in the columns of the mouse visual cortex are few when compared

with any of the other animals. The last column of Table 2 gives the mean values of the total cortical thickness (including the outermost layer) and the standard deviation of these means. These figures call for little comment except to note that the mean value for the cortex of the human precentral gyrus from these samples is low compared with the usually accepted figure of about 4 mm.

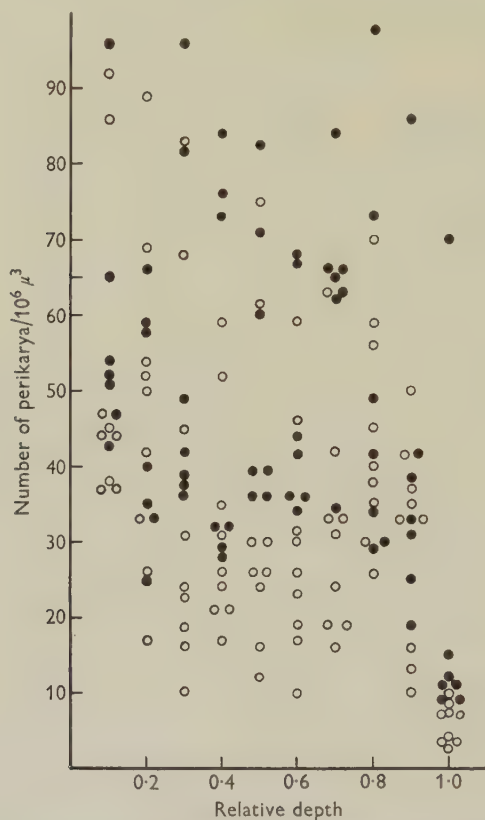


Fig. 1. The variation in neuronal density at different depths of the visual cortex in sections from two human brains. The measurements from the two brains are distinguished by black and open circles.

Table 2. *Numbers of neurons contained in a cortical cylinder with cross-sectional area 400 μ² in different regions*

Animal	Cortical region	Abbreviation	Mean number of neurons in column	Range	Mean total cortical thickness and its standard deviation
Man	Precentral gyrus	HM	23	21-25	2160 (130)
	Striate area	HV	60	47-69	1930 (150)
	Parastriate	HP	32	28-45	1780 (50)
Cat	Sigmoid gyrus	CSM	29	25-36	1790 (40)
	Post-lateral gyrus	CV	55	33-68	1520 (60)
Mouse	Visual	MV	14	13-16	680 (10)

The average neuronal densities at different depths of cortex

The publication of the whole of the original data is impracticable and they have been summarized in Table 3 which shows the mean packing density of perikarya per $10^6 \mu^3$ at different cortical depths, together with the standard deviation of these means. Generally speaking the coefficient of variation is about 10 %, but is very much smaller in parts of the human precentral cortex and in the mouse cortex. These values can be compared more easily if the mean values and their confidence limits are plotted as in Figs. 2-7. In these diagrams the mean values are shown as circles and 95 % confidence limits are marked off with bars. The extent of the limits implies that in repeated sampling the mean will lie within these limits in nineteen cases out of twenty if the distribution within the population from which the samples

Table 3. *General summary of neuronal densities at increasing depths of the cortex*

Type of cortex	No. of counts	Mean packing density of perikarya/ $10^6 \mu^3$ of cortex in terms of relative depth with corresponding standard deviation of mean									
		0.1	S.D.	0.2	S.D.	0.3	S.D.	0.4	S.D.	0.5	S.D.
Human visual (HV)	16	54.9	4.9	46.8	4.7	43.9	6.4	40.6	6.1	41.5	5.4
Human precentral (HM)	13	48.0	5.4	27.7	1.0	26.9	1.3	24.9	3.3	27.4	3.6
Human parastriate (HP)	6	76.7	8.4	66.3	5.0	55.2	5.4	43.5	3.1	56.3	8.2
Cat visual (CV)	7	124.0	11.5	95.9	6.6	72.5	4.9	78.2	10.8	76.0	4.8
Cat cruciate (CSM)	10	92.0	7.7	54.8	4.6	44.1	4.5	38.7	3.0	32.7	3.7
Mouse visual (MV)	5	86.0	3.3	81.8	0.7	77.6	3.3	78.6	4.1	52.6	3.5
		0.6	S.D.	0.7	S.D.	0.8	S.D.	0.9	S.D.	1.0	S.D.
Human visual (HV)	16	36.8	4.6	45.0	5.0	47.0	4.9	33.9	4.5	11.7	4.0
Human precentral (HM)	13	31.6	5.2	24.6	2.8	22.1	2.1	22.9	2.2	16.9	1.8
Human parastriate (HP)	6	60.0	10.1	50.2	8.1	40.3	4.5	27.7	3.3	8.7	1.9
Cat visual (CV)	7	84.2	6.5	88.7	11.8	71.2	8.9	56.9	12.5	25.9	2.7
Cat cruciate (CSM)	10	29.6	4.9	34.8	6.0	30.8	3.0	24.1	5.0	17.4	2.2
Mouse visual (MV)	5	48.4	2.1	65.2	2.4	60.6	3.9	61.2	1.3	39.2	5.8

have been drawn is normal. The wide boundaries of these limits are a measure of the hazard and difficulty involved in making general statements about the similarities and differences between different regions of cortex on the ground of the qualitative study of a few sections. In any case these limits must be treated with considerable reserve.

With this caution in mind, the mean values of the neuronal densities for the different animals may be plotted. These are shown in Figs. 8 and 9. Fig. 8 shows the mean values found for the three regions of the human cortex, Fig. 9 those for the visual cortices of the animals studied. Examination of these diagrams shows that although there may be considerable differences between the neuronal densities at any one depth of the cortex, there is a striking similarity in the forms of the graphs. Fig. 8 shows that at first the neuronal density is high and then decreases and, then, with a further increase in depth, increases to a new maximum and finally becomes very much smaller as the boundary between the grey matter and the white is reached. The similarity of the changes in neuronal density is especially clear when the parastriate (HP) and the precentral (HM) graphs are compared; if the HM graph

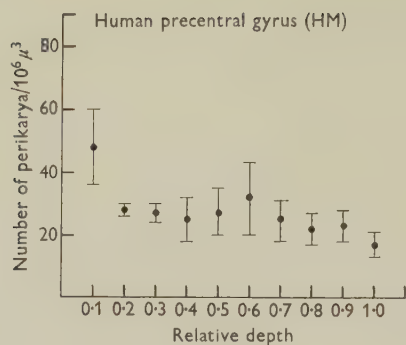


Fig. 2

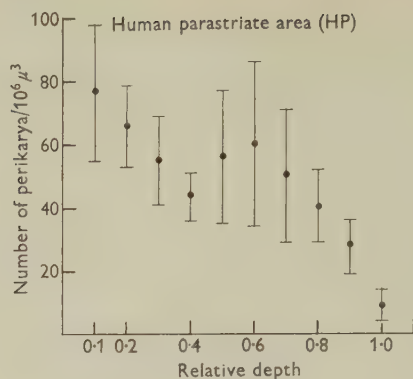


Fig. 3

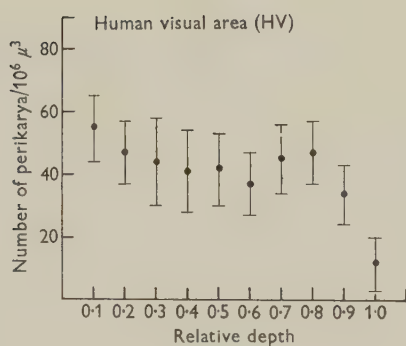


Fig. 4

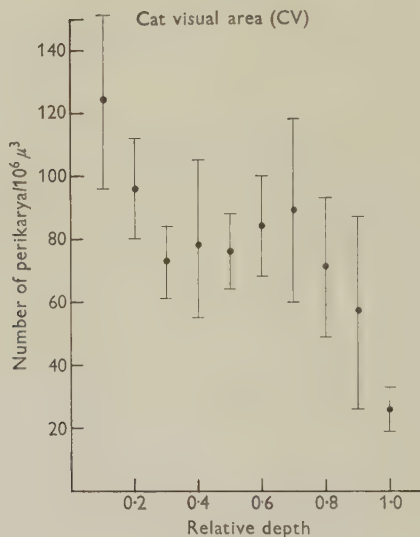


Fig. 5

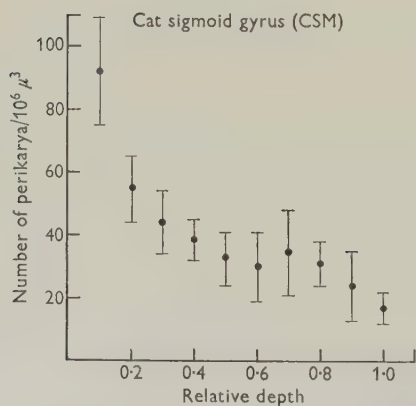


Fig. 6

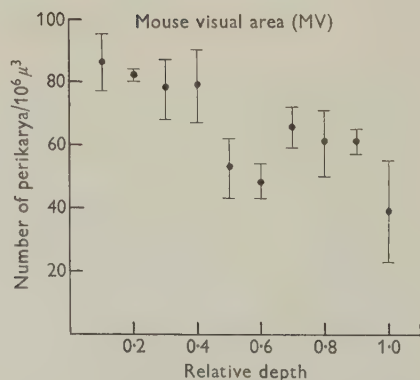


Fig. 7

Figs. 2-7. Mean values of the neuronal densities at different cortical depths with 95 % confidence limits for the various cortical regions studied.

were moved upward it would almost coincide with the HP graph. The small maximum shown at the relative depth 0.5 in the visual (HV) graph cannot be distinguished statistically from its neighbours but represents the aggregate of neurons which are assigned to various layers by the students of cyto-architectonics (to layer V by Campbell, Betz, Cajal and Meynert, to layer IVc by Brodmann and Economo and Koskinas).

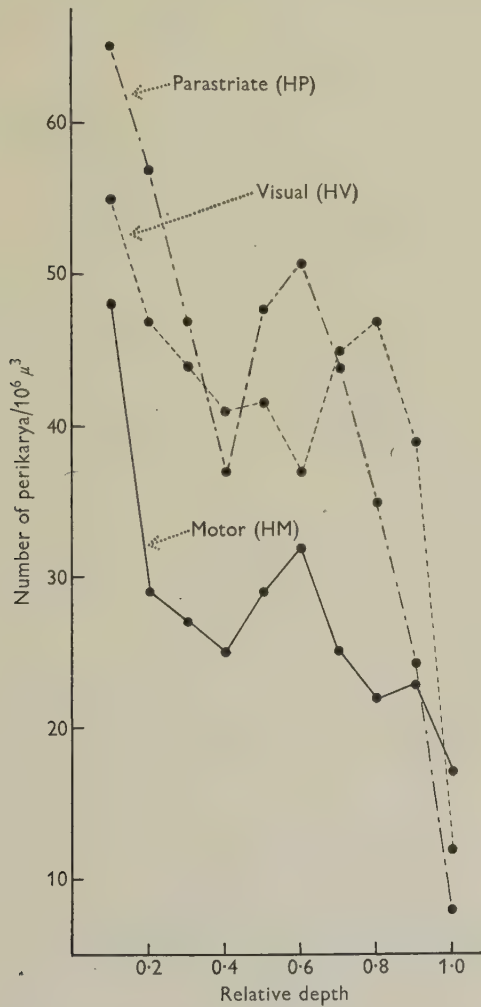


Fig. 8. Mean values of the neuronal densities at different cortical depths for three regions of human cortex.

Fig. 9 also shows the similarities between the patterns of neuronal packing in different visual cortices; again there are consistent differences between the mean densities at any one level but the patterns of density change with depth in the different cortices are similar—the rapid decrease followed by a small maximum at depth 0.4–0.5 with a second maximum deeper in the cortex.

The human precentral cortex (HM) and the cortex of the cat sigmoid gyrus show a single maximum at a depth roughly equal to that of the second maxima in the corresponding visual cortices.

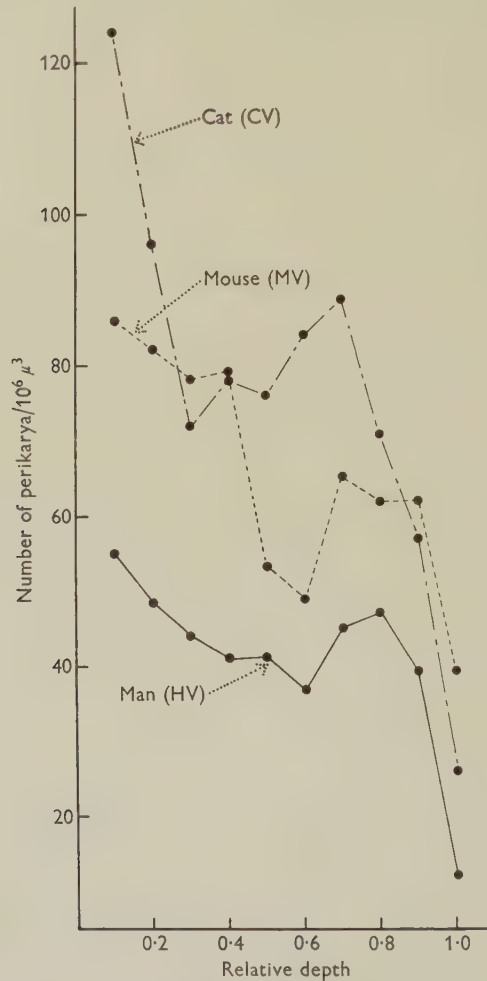


Fig. 9. Mean values of neuronal densities at different cortical depths of the visual cortices of mouse, cat and man.

The correlation between the neuronal densities at different depths of the cortex

The correlation coefficients between the neuronal densities at the various depths were calculated for each set of observations and from these different sets of coefficients the pooled correlation coefficients were computed by the standard process (e.g. Rao, 1952). Instead of quoting the coefficients it seems preferable to illustrate the results by a diagram such as Fig. 10. The relative depths are shown along the top and left-hand side of the figure and the strength of the correlation between the neuronal densities at any two depths is indicated by depth of shading in accordance

with the scheme: correlation coefficient greater than 0.5, heavy dots, between 0.25 and 0.5, small dots, 0.25 and less, left blank. It is immediately noticeable that the density at any depth is highly correlated with that at neighbouring depths: this is to be expected because there is no reason to think that the division of the cortex into ten layers of equal thickness has any biological significance and density measurements in such neighbouring layers might well be expected to be similar. One fact of interest emerges from this diagram; the densities in the lowermost quarter of the cortex are not significantly correlated with those in the outer part.

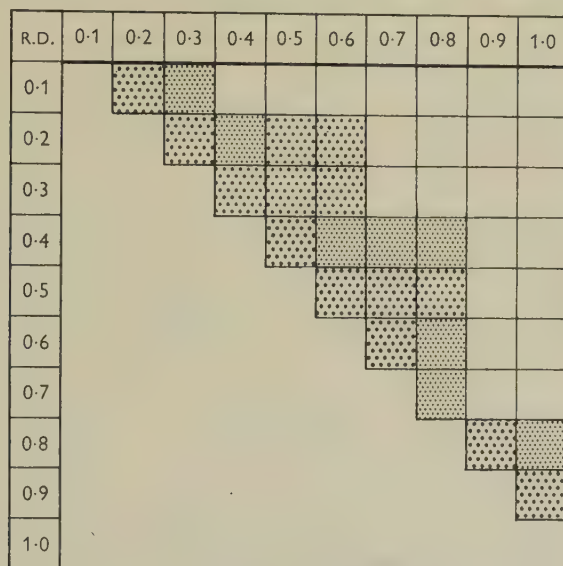


Fig. 10. Diagram to illustrate the correlation between neuronal densities at different depths. The degree of stippling of any square denotes the extent of the correlation between the densities in the two marginal 'relative depths' that form the co-ordinates of the square.

The measurement of the degree of similarity between cortices of different origin

A common problem in biological studies arises when one wishes to compare several, possibly different, groups of animals as a result of measurements of certain characters in a number of animals from each of the various groups. The choice of the characters to be measured can only be decided by the insight of the biologist; the assessment of the degree of similarity between the groups as a consequence of these measurements is a purely statistical problem.

It would be convenient if, on the basis of the measurements, a measure could be allotted to each pair of the supposed groups, which would have the properties of a 'distance'; the greater the 'distance' the more dissimilar the groups. Such a distance would have to possess certain properties which have been stated by Rao (1952) in the following way:

- (a) The distance between two groups is not less than zero.
- (b) The sum of the distance of a group from two other groups is not less than the distance between the other groups (triangle law of distance).

(c) The distance must not decrease when additional characters are considered.

(d) The increase in distance by the addition of some characters to a suitably chosen set must be relatively small so that the group constellations arrived at on the basis of the chosen set are not distorted when additional characters are considered.

The first two of these properties are mathematical and the last two are empirical in the sense that without them, the 'distance' might have little biological relevance. A suitable function of the observational measurements satisfying the four postulates was discovered by the Indian statistician Mahalanobis (1936), and is known as the Mahalanobis Generalized Distance. This function and the very lengthy computational procedure is fully described in the book by Rao (1952).

Table 4. A. *Distances when neuronal packing densities only are considered*
(10 variables)

Distance from									
HV to		HM to		HP to		CV to		CSM to	
HP	16.6	HP	24.2	HV	16.6	CSM	28.8	HV	17.9
CSM	17.9	HV	27.6	HM	24.2	HV	32.5	HP	26.2
HM	27.6	CSM	38.0	CSM	26.2	HP	33.4	CV	28.8
CV	32.5	CV	51.1	CV	33.2	MV	35.3	MV	34.5
MV	33.3	MV	52.7	MV	40.9	HM	51.1	HM	38.0

B. *Distances when absolute cortical thickness is also considered* (11 variables)

Distance from									
HV to		HM to		HP to		CV to		CSM to	
CSM	18.3	HP	24.9	HV	24.7	CSM	34.5	HV	18.3
HP	24.7	HV	30.2	HM	24.9	HV	35.8	HP	29.8
HM	30.2	CSM	38.2	CSM	29.8	HP	47.0	CV	34.5
CV	35.8	CV	57.6	CV	47.0	HM	57.6	HM	38.2
MV	54.0	MV	60.4	MV	47.2	MV	66.7	MV	51.1

In the present investigation eleven characteristics were measured on each cortical sample—ten measurements of neuronal densities at various depths and the total cortical thickness. The Generalized Distance (D) was computed using the eleven variables and also using ten variables and omitting the cortical thickness in order to see if such an omission made very much difference to the comparisons. The results are shown in Table 4A and B. It is very difficult to visualize the interpretation of these distances and they are shown diagrammatically in Fig. 11, in which each cortical region has been denoted by a small labelled circle. Any two of the regions (e.g. HV and HM) are first plotted with the distance between them drawn to scale. The line joining these two regions is used as the base-line of a set of triangles whose third vertices represent the other cortical regions studied and whose sides are proportional to the distances that are given in Table 3. For example, the lengths of the sides of the triangle HV-HP-HM are proportional to the dissimilarity between these regions and it can be seen that HP is more like HV than like HM.

Nevertheless, this type of diagram is not entirely satisfactory because it represents a three-dimensional pattern as a drawing on a plane and some of the distances will

be distorted. Since any three points in a three-dimensional space are co-planar, the vertices HV-HM-CV are co-planar and so, for example, are the vertices HV-HM-MV but this does not imply that MV and CV are co-planar and almost coincident; Table 4 shows that the distance between them is 35.3 units in a plane roughly perpendicular to the paper. Consequently Figs. 11 and 12 must only be considered as visual aids to the understanding of Table 4.

The left-hand side of Fig. 11 maps some of the distances calculated from ten variables and the right-hand side shows the corresponding distances calculated from eleven variables. The relative positions of the points are little changed in the latter

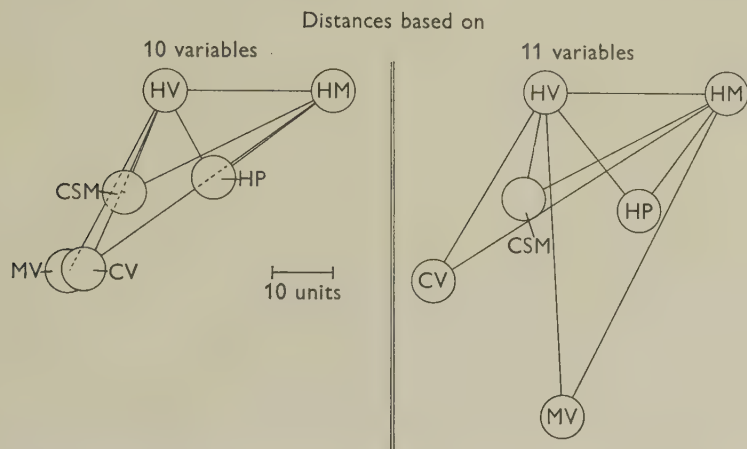


Fig. 11. Diagram to illustrate the relationship between some of the distances given in Table 4.

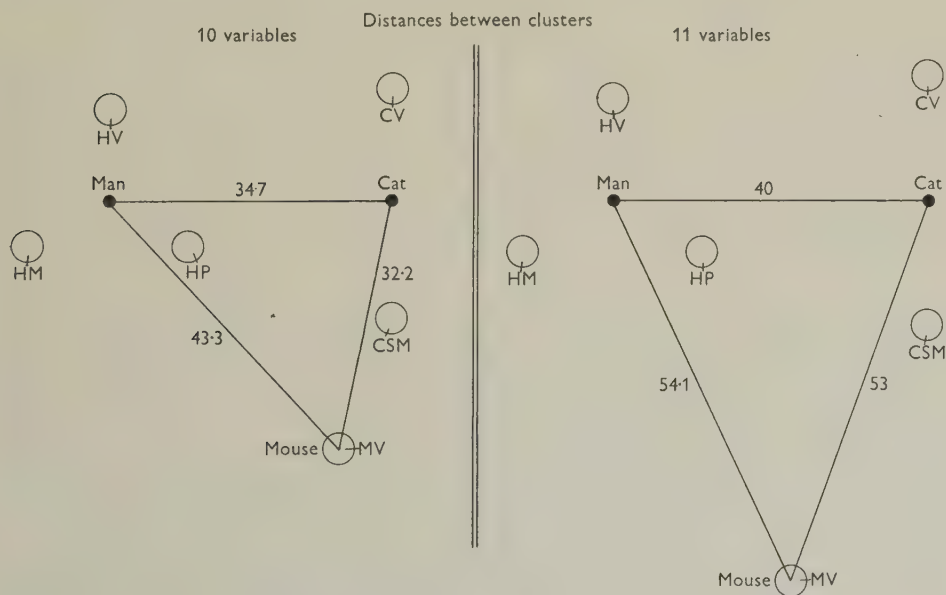


Fig. 12. Diagram to illustrate the concept of cortical 'clusters'.

case but the use of the additional parameter leads to a greater separation between the regions and especially to an increase in the distances of the mouse cortex from the other regions. In a general way it appears that as far as these variables are concerned, samples from different regions of the cortex of the same species are usually more similar to one another than are samples from cortices with similar functions from different species, e.g. HV is more like HM and HP than it is like CV or MV. This suggests that the different cortical regions from a single species form a kind of 'cluster'. This can be illustrated (Fig. 12) by calculating the mean distances between and within the 'clusters'. The general result that the distance between the cortex of man and cat is smaller than that between the cortex of man and mouse is clear and perhaps not at all surprising; but, in addition, an objective measure of this difference has been found. It is perhaps less intuitively obvious that the difference between cat and mouse visual cortex is as great as that between human and mouse visual cortex.

DISCUSSION

The neuron is the basic unit of the nervous system, and any attempt to understand the organization of this system must be interested in the packing density of these units and the patterns of interrelationship that subsist between them. The present study is only concerned with the first of these problems.

The qualitative study of the variations in neuronal density in the cerebral cortex dates back to the time of Meynert and the methods involving attempts to subdivide the cortex into areas of specific pattern with respect to perikaryal densities and sizes have been known as cytoarchitectonics for a number of years. The unsatisfactory results of these attempts were clearly shown by Lashley & Clark (1946) and, indeed, in a subsequent paper, Klotz & Clark (1950) emphasized that the variations between adult brains of the same species were so large that until the criteria for making the subdivisions were placed on an objective basis, such attempts were bound to fail. The fact remained that even an inexperienced observer could see that in many mammals the cortex associated with vision differed from that associated with motor activities. A compromise solution, still qualitative, was made by Bailey & von Bonin (1951) who made a considerable reduction in the number of distinguishable areas but also pointed out that different observers, using criteria thought to be common, did not entirely agree on the subdivisions—a conclusion similar to that of Lashley & Clark.

The only way out of this difficulty is by the use of quantitative methods and objective criteria of comparison. The present study attempts such a method but is still limited by the number of samples that it has been possible to examine. In spite of this limitation it has seemed worthwhile to indicate the kind of conclusion that further studies may justify more adequately.

An adequate study of the kind described in this paper would entail the examination of the total cortex of a number of animals of each species. At the present time such a study is impracticable and one has to select regions for study. Such a choice is not easy and the regions examined in the paper have been chosen because they are localizable by gross anatomical criteria and have some kind of well-defined activity in the living animal. The position of the cortex that receives afferent fibres from the

lateral geniculate body and is primarily associated with vision in man and cat is well-known and undisputed. One is a little less certain about the position of the visual cortex in mouse but the survey given by Haddara (1955) makes it reasonably certain that the correct region has been studied. The human parastriate region is easy to identify. If sufficiently large blocks of the occipital pole of man are sectioned in order to study the visual cortex around the calcarine fissure, the parastriate cortex is found on the sections by the sharp change in neuronal pattern that takes place at its boundary with the striate area.

Experiments with primates towards the end of the last century appeared to show that there was a sharply defined region of the cortex along the precentral gyrus that was concerned with the production of nerve impulses running to 'voluntary' muscles; this region became known as the 'motor area' and its circumscribed nature seemed to be confirmed by histological studies. This simple concept was upset by the work of Leyton & Sherrington (1917) on chimpanzees and, more recently, by the studies of Penfield & Jasper (1954) on the unanaesthetized human cortex. It became clear that there was considerable overlap between the predominantly motor function of the pre-central gyrus and the predominantly somato-sensory function of the post-central gyrus.

The situation in the cat is more confused. Some physiologists consider the cortex of the anterior sigmoid gyrus to be purely 'motor' while others consider that the cortex surrounding the cruciate sulcus, anteriorly and posteriorly, is 'sensori-motor'. A study of the literature shows that it is likely that in the lower mammals there is a true sensori-motor cortex and as the phylogenetic scale is ascended parts of the cortex adapted for somato-sensory integration become increasingly separated from parts associated with the activity of 'voluntary' muscles (van Crevel, 1958). This separation is never complete, even in man.

Considerations of the kind described in the last three paragraphs have led to the study of the cortical regions described in Table 1.

The extent of the variations in neuronal packing density in any one region has been illustrated in Fig. 1, which shows that statements based on the observations of a few sections are of little value for making general statements about these densities. Moreover, the variation with depth is not consistent from section to section of the same region of a single brain; one sample may show a higher density at some specified depth than a second sample, but a lower density at other depths. This lack of consistency is even more evident when samples from different animals are compared.

When the number of samples is increased, mean values of the neuronal densities at the different depths can be calculated together with the confidence limits for these means under the assumption of the normality of the distributions concerned.

Graphs showing the change in the mean values of the neuronal densities with depth show that the patterns of change in density are unexpectedly simple. In most of the regions of the cortex that have been studied the outermost neuron free 'layer' is followed by a region having a high neuronal density that decreases more or less sharply with the depth, reaches a minimum, increases again to a maximum and then decreases until the white matter is reached. The pattern is slightly

modified in the visual cortex where a second small maximum appears at a relative depth of about 0.4–0.5.

The reasons for this variation in density are unknown but a low neuronal density implies greater space between the perikarya. These spaces may allow additional room for blood vessels, neuroglia, dendrites and axons. Although no quantitative data are available, the density of dendritic fibres appears to be more or less uniform throughout any given region of cortex in a species with the possible exception of the outermost 'layer' containing the terminal ramifications of the apical dendrites. It is tempting to suggest that regions of lower neuronal density are associated with the presence of an increased number of axons. Such an hypothesis would suggest that the efficient operation of the cortex in higher mammals depends upon the complexity of the axonal pattern of connexions. If neuronal density is significantly related to complexity of axonal connexions, the outer minimum density would be associated with the terminal ramifications of incoming extrinsic afferent fibres and the decrease in density deeper in the cortex with the increasing number of axons leaving the cortex. The additional small maximum in the visual cortex corresponds to the aggregate of stellate neurons known to be associated with Gennari's line (Sholl, 1956; Mitra, 1955).

On the other hand, there is evidence that the neuronal density may be generally low in large animals as, for example, in the whale. Consequently, it cannot be assumed that the low neuronal density is an attribute only correlated with the efficiency of the primate cortex.

The examination of such diagrams as Figs. 8 and 9 may well lead to the conclusion that although the actual neuronal densities are different in various cortical regions, there is a basic pattern which is fundamentally similar in different regions of the cortex of the higher mammals. For example, if the HM curve of Fig. 8 were moved upwards it would approximate to the HP curve and the HV curve of Fig. 9 when moved upwards is not dissimilar to the CV curve. The problem of the interpretation of the differences in mean density between different species remains obscure even when cortical regions that are presumed to have similar functions (Fig. 9) are considered. The low neuronal density of the human visual cortex compared with that of the cat may be accounted for by the hypothesis of increased axonal density in man. The situation in the mouse then appears anomalous for there is no evidence that vision in the mouse is 'more efficient' than in the cat. It is, however, plausible that the general cortical organization in mammals low in the phylogenetic scale differs fundamentally from that in the cat and in primates. The macroscopic appearance of living cortex from mouse, rat and rabbit is much more translucent and it has a less firm consistency than the cortex in cat and the primates. These differences may indicate fundamental differences of organization.

It would be convenient if it were possible to discover reasonably simple mathematical formulae for the description and comparison of graphs such as those in Figs. 8 and 9. Various attempts were made to find such formulae and orthogonal polynomials were fitted to the data from several of the samples. The coefficients of the resulting formulae were very unwieldy and this fact, together with the impossibility of giving any biological interpretation to the polynomials, led to the abandonment of this method.

It is still desirable that some way should be found so that these various samples, each with eleven measured variables, could be compared. This type of problem was considered many years ago by Karl Pearson (Tildesley, 1921), but the Coefficient of Racial Likeness described in this paper has several disadvantages and depends upon the different variables being independent of one another. This condition is seldom fulfilled in biological investigations and is definitely unsatisfied in the present case. A different approach can be made by the use of Fisher's method of discriminant functions, but the method of Mahalanobis (1936) which has been briefly described above, was adopted.

This approach provides an objective method for estimating the relative differences between the various cortices and provides a set of numbers having the properties of Euclidean distances; the greater the difference between cortex A and cortex B, the greater the 'distance' between them. If cortex A is x units from cortex B and y units from cortex C, then the distance of cortex C from cortex B will not be greater than $(x+y)$. It must be borne in mind that the value of statements of this kind depends upon the biological insight of the investigator in the initial choice of adequate and appropriate variables, and it would be unjustifiable to regard the 'distances' given in this study as more than indications of the complete situation. No variable measuring connectivity has been considered and it is almost certain that further work will discover biochemical variations of great significance.

The present investigation nevertheless makes it possible to make certain comparisons objectively. The regions of the human cortex that have been studied are more similar to one another than they are to corresponding regions in the cat, and both human and cat cortices are more different from the mouse cortex than they are from each other. Other comparisons can be easily made from the figures given and these differences can be stated in objective and measurable terms.

The study of these cortical samples and the examinations of the data confirm the point of view (Sholl, 1956) that the cortex cannot be regarded as an organization of neurons with an invariant topographic arrangement of the perikarya. The wide variations in their density of packing suggest that the necessary invariance of pattern must be topological, maintained under changes of cortical thickness and curvature, and to be found in the connectivity pattern subsisting between the neurons and the afferent fibres to the cortex. This pattern can only be specified in statistical terms and by probability laws.

SUMMARY

1. The numbers of neuronal perikarya per unit volume ($10^6 \mu^3$) of cerebral cortex were found at increasing depths in Nissl-stained sections from selected regions of the brains of man, cat and mouse.

2. Preparations were made from formalin fixed, paraffin embedded material from adult brains of each animal. Comparable counts made by van Alphen (1945) for the human brain and by Haddara (1955) for the mouse brain, were also used.

3. The total numbers of neurons in a cylinder of cortex with cross-sectional area $400 \mu^2$ were computed. These ranged from about 30–60 in man and cat, the higher number being found in the visual cortices in each case. The number of perikarya in a similar cylinder of mouse cortex was about 14.

4. The mean values of the neuronal densities at relative depths increasing from the pial surface to the boundary of the cortex with the white matter were calculated with the corresponding standard deviations.

5. When the mean densities are plotted against relative depth, a common pattern of density change is found in the non-visual cortices showing a single minimum density at about one-third of the cortical thickness followed by a single maximum at a greater depth.

6. In the visual cortices an additional small maximum is found in the region of the stellate cell concentration at a relative depth of 0.4.

7. The neuronal densities at different cortical depths are only correlated with those at neighbouring depths.

8. The Generalized Distance of Mahalanobis was used in order to obtain a numerical measure of the degrees of similarity between the different cortices. This method showed that for the parameters studied, intraspecific cortices were more similar to one another than to cortices presumed to have similar functional activities but derived from different species.

I am very grateful to Prof. J. Z. Young for his advice and criticism; I should like to thank Miss Betty Shirra for her patient and skilful technical assistance.

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THE JUNCTIONAL REGION OF CEREBRAL HEMISPHERE AND THIRD VENTRICLE IN MAMMALIAN EMBRYOS

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In descriptions of the ontogeny of the internal capsule it is commonly stated (e.g. Hamilton, Boyd & Mossman, 1952) that the passage of nerve fibres from the cerebral hemisphere to the diencephalon is facilitated by apposition and fusion of the medial wall of the hemisphere with the lateral wall of the third ventricle (Fig. 1). Although this view has had general acceptance for many years, it would not appear to be based on unequivocal evidence. Frazer (1940) mentioned this as a possible mechanism for the passage of such fibres, but also pointed out that the same end result could be achieved by a simple thickening of the posterior wall of the interventricular foramen; he had no bias in favour of either of these two possibilities.

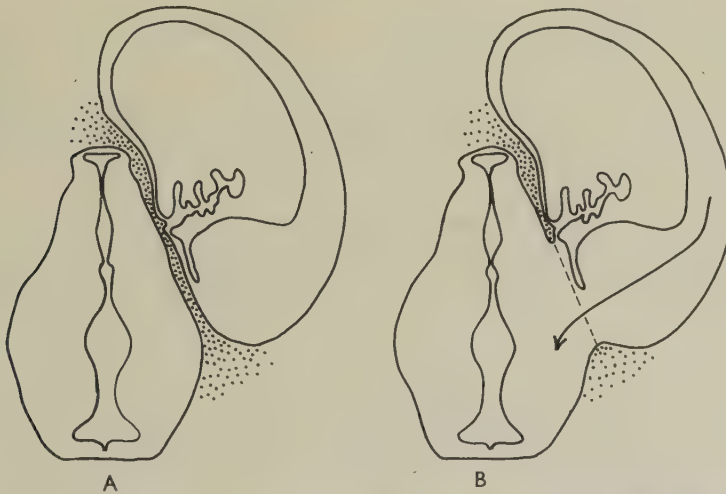


Fig. 1. Coronal sections of embryonic forebrain. A before, and B after, fusion of the medial wall of the hemisphere with the lateral wall of the third ventricle.

The nature of this problem renders it unlikely that a direct attack upon it will yield a definite answer; the obvious procedure of examining sections of the region concerned in embryos for direct evidence of fusion is inconclusive; the medial surface of the hemisphere and the lateral surface of the third ventricle are separated by a layer of vascular mesenchyme, and in the presence of such fusion it might reasonably be expected that isolated portions of mesenchyme might occasionally persist along the line of adhesion between the two surfaces. Such mesodermal rests within the central nervous system have not been observed, nor, so far as is known, have they

been reported by other workers. On theoretical grounds, it seems unlikely that two masses of nervous tissue, separated by mesoderm, could fuse with each other.

In the absence of any positive evidence for or against fusion, it is necessary to fall back on indirect or circumstantial evidence. The present work is concerned with the description of such an indirect approach and its results.

MATERIAL AND METHODS

The investigation was based on paraffin-embedded, serially sectioned early embryos of man, pig, rat and rabbit. A total of twenty-two embryos was used. In each case a graphic reconstruction was made directly on to millimetre-squared graph paper, at a linear magnification of $\times 30$ or $\times 60$; the reconstruction included the outline of one cerebral hemisphere, viewed from the medial side, together with the mid-sagittal outline of the third ventricle. Several features were outlined on the medial surface of the hemisphere.

- (1) The position of the interventricular foramen.
- (2) The extent of the area of continuity between the medial wall of the hemisphere and the lateral wall of the third ventricle, henceforward referred to as the 'Pedicule Area'.
- (3) In the larger embryos the area occupied by the thinnest portion of the medial wall of the hemisphere was delineated. This corresponds approximately to the 'Area Epithelialis' of Hines (1922), and will be referred to under the same name.
- (4) In the embryos in which the internal capsule was sufficiently evident, its size and position in the pedicle area was indicated. This was readily done as the fibres of the internal capsule at this point are invariably closely packed and occupy a roughly circular area with a well-defined edge.

On the basis of these reconstructions, the actual area occupied by (1) the fibres of the internal capsule as they pass through the pedicle area, (2) the posterior wall of the interventricular foramen in the parasagittal plane, could be estimated.

RESULTS

The reconstructions were used to study the changes in the pedicle area occurring during the early phases of development. It became clear that, for descriptive purposes, this area could be divided into four portions, centred on the interventricular foramen.

- (1) A dorsal region, forming the roof of the interventricular foramen.
- (2) A rostral region, forming the anterior wall of the foramen.
- (3) A ventral segment corresponding to the floor of the foramen, in the region of the corpus striatum.
- (4) A caudal portion, equivalent to the posterior or thalamic wall of the foramen.

The developmental changes taking place in these four divisions of the pedicle area showed general similarities in the four species studied, and it will be sufficient to describe those occurring in the human embryos.

Human 8 mm. crown rump length (Fig. 2)

The interventricular foramen is widely open; it is compressed from the ventro-caudal aspect by the primitive corpus striatum. Apart from the anlage of the corpus striatum, the pedicle area shows no features of special interest; it forms a narrow ring of tissue surrounding the centrally placed foramen.

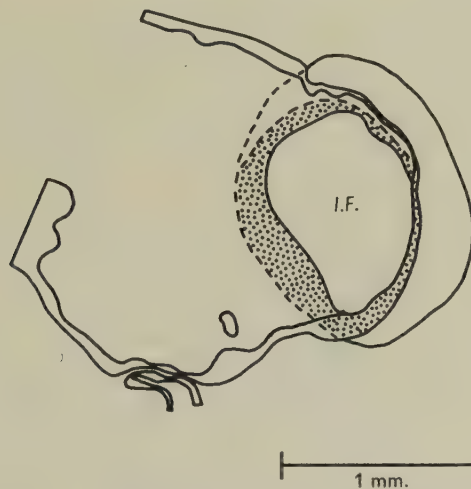


Fig. 2. Human embryo, 8 mm. C.R. length. Graphic reconstruction of forebrain. Left hemisphere seen from the medial aspect, with the mid-sagittal outline of the third ventricle superimposed. Pedicle area stippled. *I.F.* = interventricular foramen.

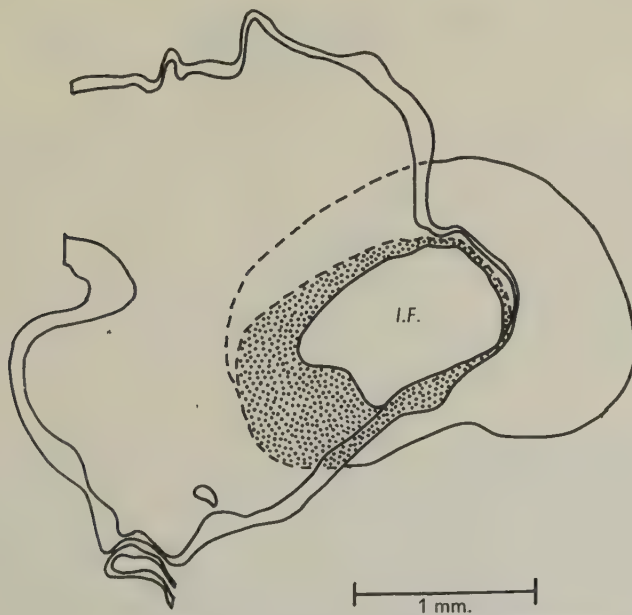


Fig. 3. Human embryo, 14.5 mm. C.R. length. Graphic reconstruction as in Fig. 2.

14.5 mm. (*Fig. 3*)

So far as the pedicle area is concerned, there has been no change in the dorsal and caudal segments. The ventral portion shows a marked increase, reflecting the growth of the corpus striatum which has taken place since the previous stage.

19.5 mm. (*Fig. 4*)

In this embryo it was possible for the first time to demarcate the 'area epithelialis' on the medial wall of the cerebral hemisphere; this takes up a roughly comma-shaped portion of the hemisphere wall, beginning rostrally at the roof of the interventricular foramen, and thence extending in a slight curve caudally, widening as it passes backwards.

The dorsal segment of the pedicle area remains unchanged. The rostral, ventral and caudal segments all display some enlargement, maximal in the case of the ventral portion, where the corpus striatum continues to grow rapidly.

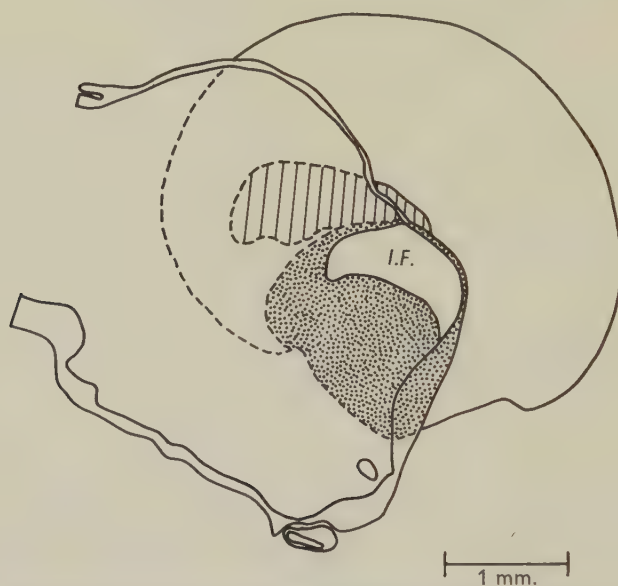


Fig. 4. Human embryo, 19.5 mm. c.r. length. Graphic reconstruction as in previous figures. Area epithelialis in vertical shading.

24 mm. (*Fig. 5*)

The outline of the cerebral hemisphere shows evidence of shrinkage and corrugation. With the extension of the hemisphere caudally, the area epithelialis has increased in length. There has been a further increase in the corpus striatum, thrusting up into the interventricular foramen. The thickening of the lamina terminalis caused by the establishment of the anterior commissure is visible in the rostral portion of the pedicle area. Again, there is no change in the dorsal portion, but the caudal region, i.e. the posterior or thalamic wall of the interventricular foramen,

displays two distinct changes; first, the internal capsule is now sufficiently advanced in development to be represented as an approximate oval within this part of the pedicle area; secondly, this segment as a whole has clearly increased in size.

It is noticeable that there is a wedge-shaped portion of the medial wall of the hemisphere intervening between the inferior edge of the area epithelialis and the caudal edge of the pedicle area.

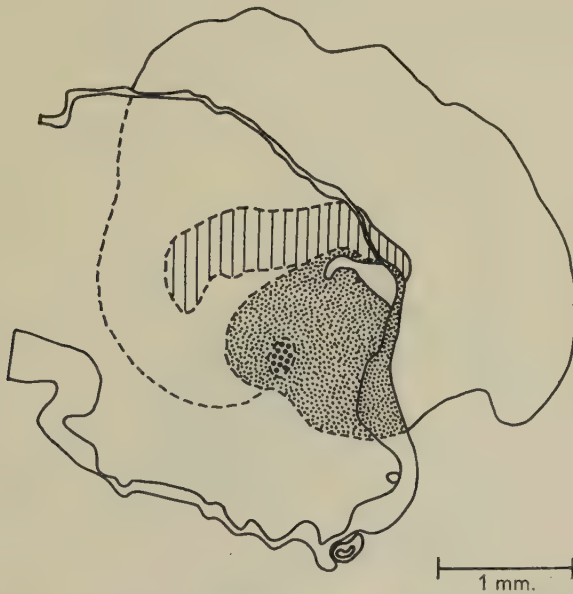


Fig. 5. Human embryo 24 mm. C.R. length. Graphic reconstruction as in previous figures. Area occupied by the internal capsule in heavy stippling.

29 mm.

The general appearance of the reconstruction based on this embryo is broadly the same as in the previous specimen, with the exception of the following.

(1) The area occupied by the posterior wall of the interventricular foramen and by the fibres of the internal capsule within it has increased.

(2) *Pari passu* with the ventro-caudal growth of the hemisphere, the area epithelialis has further extended in the same direction.

(3) The strip of hemisphere wall intervening between the caudal edge of the pedicle area and the area epithelialis has become narrower.

41 mm. (Fig. 6)

In this, the largest embryo examined in the human series, attention is directed to the fact that the area epithelialis and the caudal edge of the posterior wall of the interventricular foramen are now contiguous, the caudal segment of the pedicle area having undergone a very marked increase, so that the curved strip of the hemisphere wall which initially intervened between the two is now no longer evident.

The internal capsule now occupies a substantially greater proportion of the pedicle area, and lies in contact with the ventral edge of the area epithelialis.

In the earliest embryo examined, the interventricular foramen appeared as a wide aperture centrally placed within the pedicle area. With the growth of the corpus striatum in the ventral segment and the expansion of the caudal wall of the foramen, the foramen itself has gradually undergone an apparent shift, and now lies in the extreme dorso-rostral angle of the pedicle area. The dorsal segment of the area, forming the roof of the foramen, has remained unchanged.

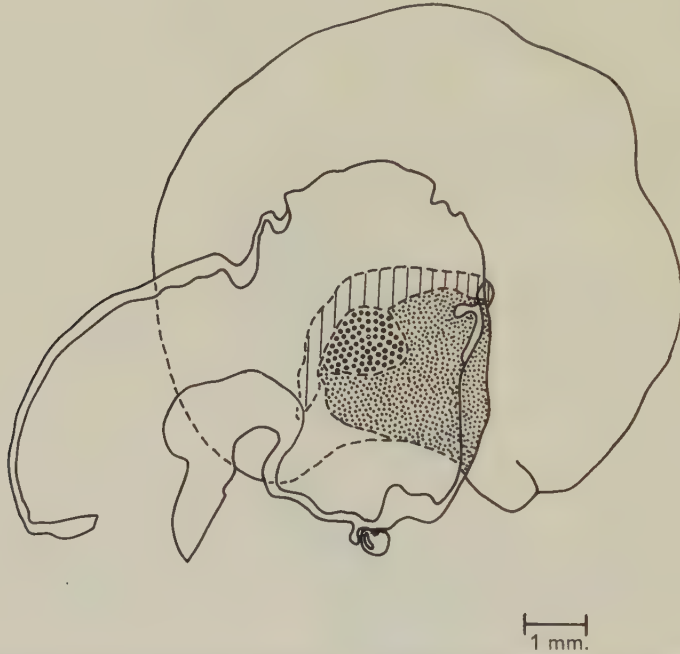


Fig. 6. Human embryo, 41 mm. C.R. length. Graphic reconstruction as in previous figures.

Measurements within the pedicle area

The actual areas, in square millimetres, of the caudal region of the pedicle area and of the enclosed fibres of the internal capsule are presented in Table 1.

These measurements are expressed graphically in Figs. 7 and 8. The graphs based on the rat, rabbit and pig embryos resemble each other quite closely, each being a roughly sigmoid curve. Since the gradient of the curve is a measure of the rate of expansion of the area under consideration, it can be stated that in these three species there are three phases of growth detectable in the posterior wall of the interventricular foramen; there is an initial period of relatively slow growth, followed by a phase of much more rapid increase, which in turn gives way to a third phase in which growth is again slower. The commencement of the second phase can be related approximately to the C.R. length as follows: pig, 30 mm.; rat, 10 mm.; rabbit, 15 mm.

In the series of human embryos examined only the first and second periods of growth occur, but here again there is clear evidence that an initial episode of

relatively slow expansion of the caudal portion of the pedicle area is succeeded quite suddenly at about 25 mm. c.r. length by a phase of much more rapid growth, which persists up to, and possibly beyond, the end of the series.

Table 1

c.r. length (mm.)	Area of posterior wall of inter-tricular foramen (mm. ²)	Area of internal capsule (mm. ²)
Human		
8	0.19	Nil
14.5	0.41	Nil
19.5	0.54	Nil
24	0.73	0.03
29	1.14	0.11
41	4.10	1.18
Pig		
13	0.18	Nil
17.5	0.27	Nil
29	0.75	0.18
36	1.62	0.41
42.5	1.78	0.40
Rat		
5	0.04	Nil
11	0.20	Nil
15	0.60	0.10
19	0.70	0.12
22	0.75	0.08
30	0.87	0.15
Rabbit		
8	0.15	Nil
10	0.14	Nil
16	0.28	Nil
20	0.55	0.09
30	0.79	0.29

The fibres of the internal capsule occupy a clearly circumscribed area, in a parasagittal plane, at the junction of cerebral hemisphere and third ventricle. They are first present in sufficient numbers to be detectable at the following stages in the four species (c.r. length): man, 24 mm.; pig, 29 mm.; rat, 15 mm.; rabbit, 20 mm.

In the 24 mm. human embryo they occupy an area of only 0.03 mm.² suggesting that the internal capsule in man probably makes its first appearance very shortly before this. Cooper (1946) states that the internal capsule is first established in human embryos of seven weeks (22 mm. c.r. length), and the present work would appear to confirm this. So far as pig, rat and rabbit are concerned, the measurements of the area occupied by these fibres show that the internal capsule is already comparatively well developed at 29, 15 and 20 mm. c.r. length, respectively, and it is likely that the actual initiation of the internal capsule occurs at a slightly earlier

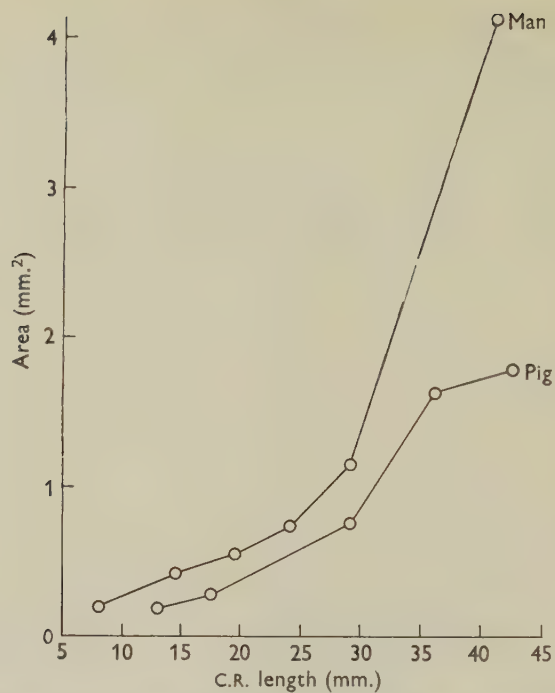


Fig 7 Measurements of the area of the posterior wall of the interventricular foramen in man and pig plotted against c.r. length.

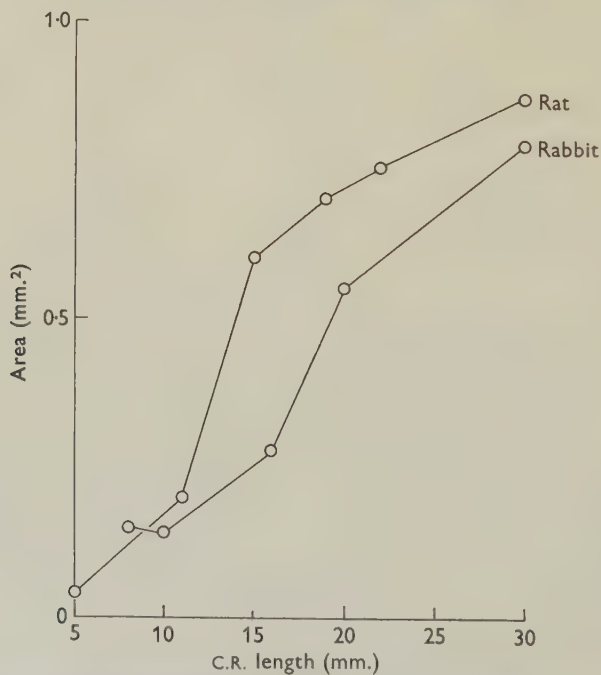


Fig. 8. Measurements of the area of the posterior wall of the interventricular foramen in rat and rabbit plotted against c.r. length.

stage of development in each case. Rose (1942) mentions 21 mm. C.R. length as the stage at which these fibres first appear in the rabbit; the present work suggests that this may in fact occur a little earlier. In the pig and rat it may be said that the internal capsule is probably initially formed just prior to 29 and 15 mm. C.R. length, respectively.

DISCUSSION

As already pointed out, in the absence of any positive evidence for or against the occurrence of fusion between the medial wall of the cerebral hemisphere and the lateral wall of the third ventricle, recourse must be made to indirect or circumstantial evidence. It is believed that such evidence is provided by this investigation.

In the first place, study of the graphic reconstructions (Figs. 2-6) reveals that the initiation of the internal capsule is associated with a caudal expansion of the posterior wall of the interventricular foramen; the posterior edge of the pedicle area is at first separated from the anterior edge of the area epithelialis by a strip of relatively undifferentiated hemisphere wall, but as the pedicle area extends backwards the two gradually come together and ultimately are in complete edge-to-edge contact. This sequence of events can be followed in each of the species studied. This caudal expansion of the posterior wall of the foramen could be the result of either (1) a thickening of the wall, or (2) fusion of the medial wall of the hemisphere with the lateral wall of the diencephalon. However, the estimations of the cross-sectional area (in a parasagittal plane) of the posterior wall of the interventricular foramen, taken in conjunction with the time of formation of the internal capsule, bring out the fact that the phase of most rapid growth of the posterior wall synchronizes with the appearance and multiplication of internal capsule fibres crossing the di-telencephalic junction. Thus a comparison of the time of onset of the phase of rapid growth of the posterior wall with that of the appearance of the internal capsule (Table 2) suggests that the two events are related.

Table 2

	Onset of rapid growth of posterior wall of foramen (mm. C.R. length)	Appearance of internal capsule (mm. C.R. length)
Man	25	24
Pig	30	29
Rat	10	< 15
Rabbit	15	< 20

If the pathway for the internal capsule was established by fusion of the cerebral hemisphere with the diencephalon, such fusion would necessarily precede the establishment of the internal capsule, whose fibres would clearly be unable to penetrate the mesoderm-filled interval between the two surfaces concerned. Preliminary fusion of this kind would give rise to a period of rapid expansion of the posterior wall of the interventricular foramen which would take place *before* the appearance of the internal capsule.

It has been shown here that, certainly in the cases of man and pig, less obviously in rat and rabbit, rapid expansion of the posterior wall of the foramen coincides in onset with the establishment of the internal capsule.

Thus the weight of the evidence considered here is in favour of a simple thickening of the caudal portion of the pedicle area, i.e. of the thalamic wall of the interventricular foramen, rather than of a process of fusion of adjacent surfaces, as the basis of the pathway for the internal capsule.

SUMMARY

1. Graphic reconstructions of the forebrains of early embryos of man, pig, rat and rabbit have been employed to study qualitatively and quantitatively the changes taking place at the di-telencephalic junction during the establishment of the internal capsule.

2. The initial formation of the internal capsule is associated with a rapid expansion of the posterior wall of the interventricular foramen.

3. The results obtained suggest that a pathway is provided for the internal capsule, between the cerebral hemisphere and the third ventricle, not by fusion of adjacent surfaces, but rather by a true thickening of the posterior wall of the interventricular foramen.

This investigation was undertaken at the suggestion of Prof. A. Durward, and has been supported throughout by his interest and advice.

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THE MAMMALIAN CAUDATE NUCLEUS

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INTRODUCTION

The human caudate nucleus, particularly its head, develops from three successive parts (Hewitt, 1958). The first or medial striatal elevation is succeeded on its outer side by the lateral striatal elevation. Finally the intermediate striatal elevation develops between the other two and forms the majority of the head, enveloping the lateral elevation in the process.

It is a reasonable supposition that this cycle of events reflects the elaboration of the nucleus which has occurred during the course of its evolution. To investigate this hypothesis, the nucleus has been examined in a number of mammals with the hope that a better understanding of its function will be gained.

MATERIALS AND METHODS

Unless otherwise stated, one cerebral hemisphere from each of the following mammals has been examined:

Monotremata

- Platypus (*Ornithorhynchus anatinus*)
- Spiny anteater (*Tachyglossus aculeatus*)

Marsupialia

- Two pouched mice (*Sminthopsis crassicaudata*)
- Short nosed bandicoot (*Perameles gunni*)
- Red-necked wallaby (*Wallabia rufogrisea*)
- Tree kangaroo (*Dendrolagus* species)
- Ring-tailed possum (*Pseudocheirus convolutor*)

Insectivora

- Hedgehog (*Erinaceus* species)

Xenarthra

- Six-banded armadillo (*Dasypus sexcinctus*)

Cheiroptera

- Indian fruit bat (*Pteropus giganteus*)

Rodentia

- Grey squirrel (*Sciurus carolinensis*)
- Crestless porcupine (*Hystrix javanica*)

Lagomorpha

- Six rabbits (*Oryctolagus auriculus*)

Artiodactyla

Sheep (*Ovis aries*)Yellowbacked duiker (*Cephalophus sylvicultrix*)Musk deer (*Moschus moschiferus*)

Carnivora

African genet (*Genetta species*)Dog (*Canis familiaris*)Common fox (*Vulpes vulpes*)Six cats (*Felis domestica*)European otter (*Lutra lutra*)Walrus (*Odobenus rosmarus*)

Primates

Malayan tree shrew (*Tupaia belangeri*)Brown lemur (*Lemur fulvus*)Common marmoset (*Hapale jacchus*)Black-tailed marmoset (*Hapale argentata*)Lion marmoset (*Leontocebus rosalia*)Six macaques (*Macaca species*)Langur (*Semnopithecus leucoprymnus*)Black and white colobus monkey (*Colobus species*)Gibbon (*Hylobates lar*)Orang-outang (*Simia satyrus*)Chimpanzee (*Anthropithecus troglodytes*)Man (*Homo sapiens*)

The caudate and amygdaloid nuclei in each cerebral hemisphere were exposed by removing the roof and medial wall of the ventricle. Usually a portion of the thalamus was also removed to improve exposure of the nuclei.

RESULTS

The nuclei of the rabbit, cat and macaque are considered in some detail first, because they represent well-defined evolutionary types. The nuclei of the remaining animals can then be compared with this pattern and the interpretation of the findings becomes much clearer and easier to follow. Accordingly, the nuclei of these other animals are described in less detail last.

In the rabbit, the head of the caudate nucleus is small relative to the moderately broad tail into which it smoothly tapers posteriorly (Pl. 1, figs. 5, 6). From side to side the head is narrow, especially anteriorly, whereas posteriorly it becomes broader. On its medial or ventricular aspect the head of the nucleus is subdivided into two parts. The medial part (Pl. 1, figs. 5, 6, *M.S.E.* and *E.*), tapers towards the inter-ventricular foramen and is continuous behind with the tail of the nucleus (Pl. 1, fig. 5, *T.*). The lateral part (Pl. 1, figs. 5, 6, *L.S.E.*), is subdivided into two by an oblique ridge. In front of the ridge it forms a broad rim around the front of the rest of the head and joins the septal region below and in front of the medial part over the region of the olfactory tubercle. Behind the ridge, the lateral part tapers to the outer edge of the tail and its surface changes direction to face upwards and come into

contact with the roof of the ventricle. When viewed from above (Pl. 1, fig. 6) the outer edge of the head is in the same plane as the outer edge of the tail. The tail is of even width and posteriorly joins the long oval flattened amygdaloid nucleus (Pl. 1, fig. 5, *T.* and *A.*). From this nucleus spring the fibres of the stria terminalis which extends along the inner border of the nucleus, in the deep groove between it and the thalamus, to the interventricular foramen.

In the cat, the outstanding feature of the head of the nucleus (Pl. 1, fig. 7) is its large size compared with the tail which is so narrow that it is almost imperceptible in places. The difference between the two is accentuated by the abrupt transition between them. When compared with that of the rabbit the head of the nucleus is relatively large, especially in its transverse width. Whereas the outer border of the head of the nucleus in the rabbit is in the same line as the outer margin of the tail, the outer border of the head in the cat is expanded laterally, making this border of the whole nucleus somewhat sinuous. The medial part of the nucleus (Pl. 1, fig. 7, *E.*), tapering into the interventricular foramen, can clearly be distinguished and remains undiminished in size, relative to that of the rabbit. It is still separated from the outer part of the head (Pl. 1, fig. 7, *L.S.E.*) by a rounded border, and the whole surface of this lateral part faces upwards. No specific lateral or intermediate parts of this outer region of the head can be distinguished. When compared with the rabbit this part of the head is larger, due to its increase in width, because it has been expanded sideways with the result that its outer border becomes convex. In front, this outer part forms a rounded ridge curving medially and downwards around the rest of the head to join the septal region in front of the anterior commissure above the olfactory tubercle. The tail of the nucleus is only clearly distinguishable at its posterior extremity where it merges with the well-defined amygdaloid nucleus (Pl. 1, fig. 7, *A.*). The stria terminalis is still present as a thin band between the amygdaloid nucleus and the interventricular foramen lying in the still deep gutter between the thalamus and the nucleus.

In the macaque, although the head of the nucleus is large and the tail is thin (Pl. 1, fig. 9), nevertheless, the transition between them is smoothly effected. Unlike that of the cat the outer border of the head and tail is in a straight line. In front the head ends in a straight border. The forward extent of the head of this nucleus is difficult to assess in relative terms. The anterior commissure (Pl. 1, figs. 5-7 and 9, *A.C.*), probably provides the best fixed point about which a relationship may be established, and in the macaque a smaller proportion of the whole nuclear head lies anterior to the anterior commissure than in the cat or rabbit. From its medial aspect the head is triangular in outline with a flat medial surface which abuts against the septum lucidum. This surface is continuous below with a thin triangular strip, the apex of which enters the interventricular foramen in conjunction with the stria terminalis running along its posterior border (Pl. 1, fig. 9, *A.* and *S.T.*). The smooth, slightly convex upper surface of the head is at right angles to the medial surface from which it is separated by a rounded border. In front this border divides below to enclose a small, flattened triangular area continuous below with the septal region. The tail of the nucleus is visible in its whole extent as a faint narrow band becoming wider just before its junction with the amygdaloid nucleus from which it is separated by a groove (Pl. 1, fig. 9, *A.*). The stria terminalis forms

a distinct band, particularly at its commencement, in the now shallow groove between the nucleus and the thalamus.

In the two monotremes the nuclei show marked differences (Pl. 1, figs. 1, 2). In the platypus the head of the nucleus is flattened from side to side and is continuous behind with a narrow tail. The ventricular surface of the head is flattened or faintly concave and tapers into the interventricular foramen (Pl. 1, fig. 1, *E.*). In the spiny anteater the nucleus is relatively larger. The head is divided into two by a wavy border produced by the impress of the cortical sulci, which, in this brain, produce ridges on the wall of underlying ventricle. The part of the head medial to this resembles the whole head of the nucleus in the platypus. The part immediately medial to this border and all that lateral to it forms a broad rim around the outer side of the head (Pl. 1, fig. 2, *L.S.E.*). Below and in front, this part becomes wider and joins the floor of the ventricle over the olfactory tubercle. The whole head resembles that of the platypus with this outer rim superadded. Behind, the head is continuous with a broad tail which is separated from the thalamus by a deep groove and joins a well-defined amygdaloid nucleus.

Although there are small differences between the caudate nuclei in the marsupial brains examined, that of the wallaby can be regarded as a typical example (Pl. 1, fig. 3). The head resembles that of the spiny anteater and, in some ways, that of a 180 mm. human foetus. It is large compared with the size of the cerebral hemisphere, and extends well in front of the large anterior commissure (Pl. 1, fig. 3, *A.C.*). As in the spiny anteater, the head is divided into two surfaces by a sinuous border but the surface lateral to this is narrow and is separated from the outer wall of the hemisphere by a shallow groove. The medial surface is subdivided by a groove running downwards and forwards. The part medial to this tapers into the interventricular foramen (Pl. 1, fig. 3, *E.*) and is continuous behind with the thin tail (Pl. 1, fig. 3, *T.*). The part lateral to the groove (Pl. 1, fig. 3, *L.S.E.*), together with the rest of the head forms, as before, a rim around the head which becomes quite bulky in front and merges with the rest of the head over the olfactory tubercle. The amygdaloid nucleus (Pl. 1, fig. 3, *A.*) is large and well defined.

In the six-banded armadillo the head of the nucleus has some resemblance to that of the rabbit but it has the particular feature that the outer border projects backwards as a spur which blends with the side wall of the hemisphere.

The head of the nucleus in the hedgehog resembles that of the armadillo except that its ventricular surface is more convex. The spur-like backward projection is again present. Below it is a small tubercle in which the short tail of the nucleus ends. Behind and below this, separated from it by a groove, is a second tubercle from which the stria terminalis arises. This form of the amygdaloid complex somewhat resembles that of the macaque.

Apart from the slightly larger and more convex head of the nucleus in the grey squirrel and the crestless porcupine, it is almost identical, in form, with that of the rabbit. This appearance of the nucleus also applies to that of the indian fruit bat.

In none of the artiodactyla or the other carnivores did the form of the nucleus display any features differing from those in the cat.

The caudate nucleus in the primates varies in form. In the malayan tree shrew (Pl. 1, fig. 4) the form of the head of the nucleus is intermediate between that of the

rabbit and cat. The head is similar to that of the rabbit except that its outer border is slightly convex and it is not quite in line with the outer border of the tail. In this fashion it has some of the appearance of the head in the cat but it is neither so large nor is its junction with the thin short tail so abrupt. In the lemurs (Pl. 1, fig. 8), the nucleus is very similar to the carnivores, whereas in the chimpanzee (Pl. 1, fig. 10) the nucleus has almost the appearance of that in the human brain (Pl. 1, fig. 11). The macaque and the other primates examined represent intermediate forms.

DISCUSSION

The interpretation of the functional significance of the differences between the various caudate nuclei examined is difficult. At the outset, it was hoped that the three components seen in the developing human caudate nucleus would be clearly discernible in some mammals, especially the more lowly. Although these parts cannot be clearly and sharply distinguished, nevertheless their existence can be appreciated sufficiently for some conclusions to be drawn.

In all the animals examined the medial striatal elevation is present and is represented by the part of the nucleus extending into the interventricular foramen (Pl. 1, figs. 1-9 and 11, *M.S.E.* and *E.*). In animals other than primates it is relatively large and most of the tail seems to be a backward continuation of it. In the primates its extent becomes less and less distinct as the scale is ascended, but most of the tail is still continuous with the medial surface of the head and the medial elevation may well be still quite large. This is, to some extent, confirmed by the amount of this part remaining in the human caudate nucleus in the final stages of its development (Hewitt, 1958).

Although the presence of an intermediate striatal elevation cannot be discounted in the nucleus of the rabbit, the majority of the remainder of the nucleus in this animal represents the lateral striatal elevation forming a broad rim around the outer side of the head (Pl. 1, figs. 5, 6, *L.S.E.*). The form of the head of the nucleus in the cat could indicate the insinuation of an intermediate elevation between the two parts of the head of the rabbit nucleus. This would explain the broad head of the cat's nucleus with a convex outer border no longer in line with the tail as it is in the rabbit. In the macaque the head is still broad but the outer border is once more in line with the tail and the head does not extend so far forwards. This state of affairs could result from some increase in size of the intermediate elevation and an absence or diminution of the lateral elevation.

The reason for these changes in the form of the nucleus becomes more understandable, perhaps, if the functions of the components are considered. Some authors have suggested that the medial elevation in the developing human nucleus represents a part concerned with olfaction. That this part is the first to appear in development is the only justification for this conclusion. This theory is difficult to reconcile with the persistence of a medial elevation in man and other primates, all of which are microsmatic. There would be just as much reason for believing it to be concerned with visceral activities because it tapers through the interventricular foramen to the hypothalamus. If, however, the lateral elevation is concerned with olfaction, this would account for its presence in the rabbit and cat, both of which

are macrosomatic, its reduction in the primates and its transient appearance in the developing human nucleus.

The brains of the rabbit, cat and macaque possess two other features which may have a bearing on the function of these components. The convoluted cerebral hemisphere of the cat and macaque are in marked contrast with the smooth hemisphere of the rabbit. The thalamus in the cat and macaque is more extensive than in the rabbit as evidenced by the depth of the groove between the nucleus and the thalamus. The presence and growth of an intermediate striatal elevation in the cat and macaque would not, therefore, be unexpected if it is interconnected with the thalamus and/or cerebral cortex.

The functional explanation for the form of the nucleus is also supported by the form of the nuclei in the other brains examined and those of the platypus and spiny anteater are particularly helpful. It has already been mentioned that the caudate nucleus of the spiny anteater closely resembles that of the platypus with an outer rim superadded. This rim represents the lateral striatal elevation and, if it is functionally concerned with olfaction, its absence is explicable in an aquatic and probably microsmatic animal, such as platypus. The appearance of the nucleus in the highly osmotic marsupials also lends support to the idea that the lateral elevation is concerned with olfaction because it forms the large outer part of the head lateral to the groove on the surface. The slightly expanded head of the nucleus in the tree shrew is presumably due to the early growth of the intermediate elevation which is not as advanced as in the lemur. Both these animals are macrosomatic and the presence of all three elevations in the head of the nucleus of each accounts for its resemblance to that of the cat.

The composition of the tail of the nucleus remains uncertain. When its development was considered the lateral elevation seemed to provide the major contribution. This is supported by the form of the tail of the nucleus in the two monotremes. The broad tail in the spiny anteater could be explained by the contribution it receives from the lateral striatal elevation which is larger than in the platypus in which the tail is narrower. In the rabbit and other lower mammals, however, it appears to be the medial elevation with which most of the tail is continuous. The junction between the head and tail of the nucleus in the cat and lower primates is abrupt compared with that of the higher primates, in which the head tapers smoothly into the tail. This can only be explained as the outcome of the backward growth of the intermediate elevation which is developing at this stage. Thus the tail would ultimately seem to be composed of some of all three components.

The form of the amygdaloid nucleus in most of the brains examined conforms to a regular pattern. The exception to this is in the hedgehog where two tubercles are present at the end of the short tail of the nucleus. The appearance in the armadillo seems to be a simple form of this. The reason for these appearances is unknown. It cannot necessarily be regarded as an early stage of the primate amygdaloid complex because, although the tail in the macaque expands before joining the amygdaloid nucleus, this is not seen in the tree shrew and lemur nor is it seen in the developing human nucleus where it might also be expected if this is true.

SUMMARY

1. The form of the caudate and amygdaloid nuclei has been examined in a number of mammals, particularly the rabbit, cat and macaque.

2. The caudate nucleus is composed of varying proportions of the three components previously described in the developing human nucleus. In lower mammals the head of the caudate nucleus is largely composed of two striatal elevations, a medial and a lateral. In higher mammals the expansion of the head of the nucleus is thought to be due to the increasing size of a third or intermediate striatal elevation. The form of the nucleus in the primates is explained by the steady reduction in size of the lateral elevation which ultimately becomes enveloped by the growing intermediate elevation.

3. An interpretation of the functional significance of these components has been attempted in order to explain the changes occurring in the form of the mammalian caudate nucleus. It is suggested that the medial elevation is concerned with visceral activities. The large size of the lateral elevation in macrosmatic animals and its reduced size in microsmatic animals would be explained on the grounds that it is concerned with olfactory functions. The intermediate elevation develops and enlarges at the same time as the thalamus and cerebral cortex are expanding, which suggests that perhaps all three structures may be interconnected.

4. The contribution made by the three striatal elevations to the form of the tail of the caudate nucleus is uncertain. All three components appear to make some contribution in higher mammals, but the proportion of each is difficult to assess.

5. The form of the amygdaloid nucleus conforms to a regular pattern similar to that in man. The only differences are those of size, which is related to the overall size of the caudate nucleus and in the hedgehog and macaque where two tubercles are present.

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REFERENCE

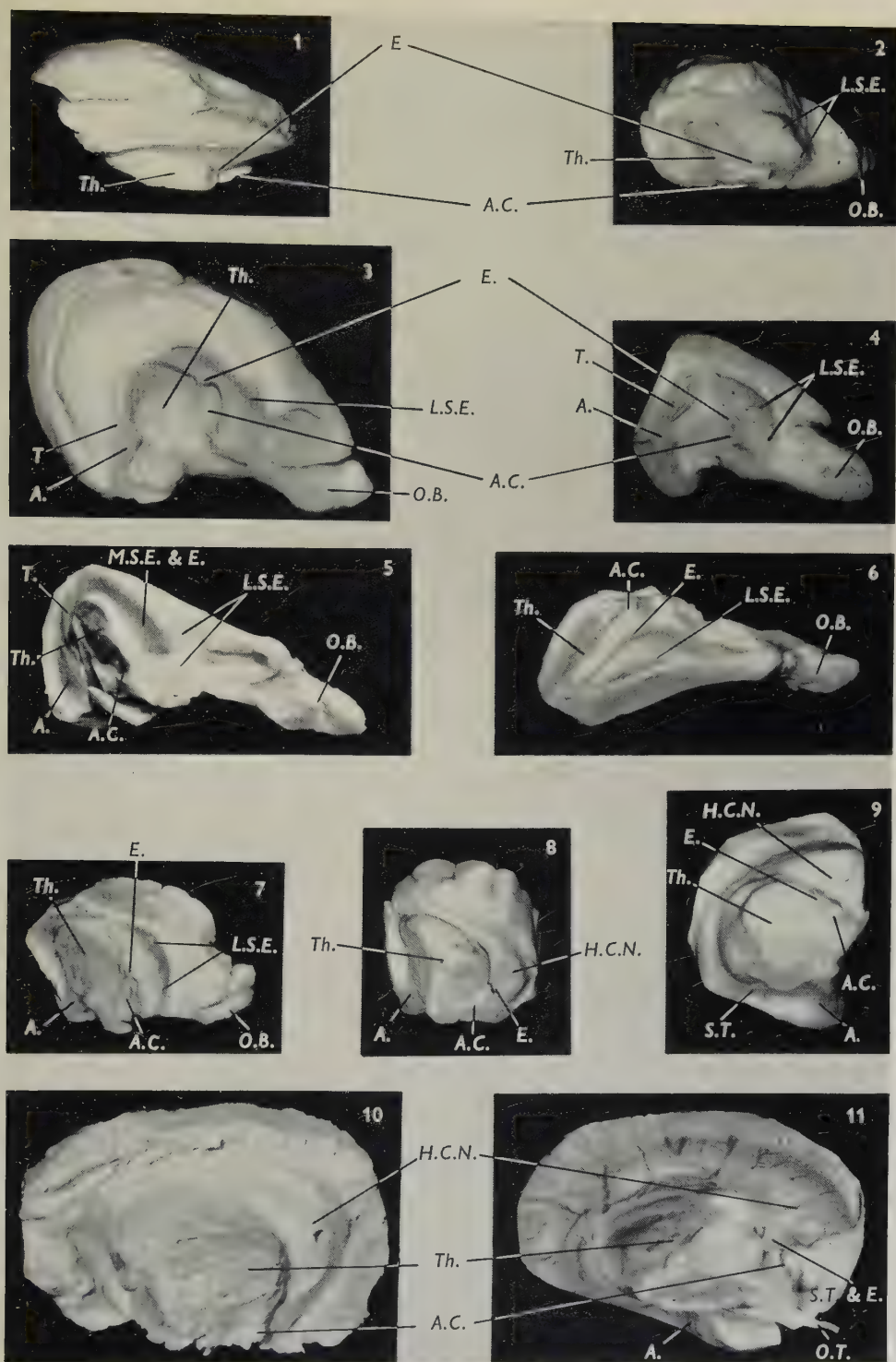
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EXPLANATION OF PLATE

Left cerebral hemisphere of some mammals in which the medial wall and roof of the ventricle and part of the thalamus have been removed to expose the caudate and amygdaloid nuclei. All are viewed from the medial aspect except Figs. 1 and 6 which are viewed from above. With the exception of Fig. 11, which is half natural size, the remainder are natural size.

- | | |
|-----------------------------|----------------------|
| Fig. 1. Platypus. | Fig. 7. Cat. |
| Fig. 2. Spiny anteater. | Fig. 8. Brown lemur. |
| Fig. 3. Red-necked wallaby. | Fig. 9. Macaque. |
| Fig. 4. Malayan tree shrew | Fig. 10. Chimpanzee. |
| Figs. 5, 6. Rabbit. | Fig. 11. Man. |

The abbreviations used signify: *A.*, Amygdaloid nucleus; *A.C.*, anterior commissure; *E.*, extension of medial striatal elevation through the interventricular foramen; *H.C.N.*, head of caudate nucleus; *L.S.E.*, lateral striatal elevation; *M.S.E.*, medial striatal elevation; *O.B.*, olfactory bulb; *O.T.*, olfactory tubercle; *S.T.*, stria terminalis; *T.*, tail of the caudate nucleus; *Th.*, thalamus.



EXPERIMENTAL STUDIES ON THE STAINING OF NERVOUS TISSUE WITH SILVER PROTEINATES

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INTRODUCTION

Soluble silver proteinates were first synthesized by Eichengrün in 1898 (Porter & Davenport, 1951) and Regaud & Dubreuil (1903) used them, in particular Protargol, to stain epithelia in fresh tissue. In the following year, Lugaro (1904) employed silver proteinates to stain neurofibrillae and in 1933, Bartelmez & Hoerr substituted Protargol for the silver nitrate used in Rogers' (1931) method of staining nerves in paraffin sections. It was not until 1936 that Protargol gained wide popularity as an histological reagent for nerve staining by the introduction of Bodian's method, in which the addition of metallic copper was used to improve the selectivity of the stain for nerves.

Between 1936 and the beginning of the last war, various methods of using Protargol were suggested (see Davenport & Kline, 1938; Davenport, MacArthur & Bruesch, 1939; and Davenport, Porter & Thomas, 1947), and although many proteinates were available commercially, only Protargol manufactured by Bayer in Germany and Winthrop in America was satisfactory for staining nerves. After 1939, when Bayer Protargol was no longer available, a number of methods employing simple silver salts were published, but despite attempts to simplify and investigate these latter methods of staining (see Silver, 1942; Holmes, 1943; Palmgren, 1948; Romanes, 1950; Samuel, 1953; Peters, 1955; and Wolman, 1955) they proved to be less satisfactory and frequently less consistent than the Bodian technique. More recently Protargol-S has been produced by Winthrop-Stearns Inc. of America, and, in 1956, Polley described a new silver compound from France (Laboratoire Roque); both of these compounds have been widely used to stain nerves.

Some of the factors involved in staining with silver proteinates were considered by Bodian (1936), Holmes (1943), Romanes (1950), and Davenport and his co-workers. Bodian (1936) found that when sections were impregnated in Protargol solutions which contained metallic copper, copper went into solution and some was deposited, along with silver, on nerve fibres. He also suggested that since silver was plated-out on to the copper, it was likely that the ionic silver content of the Protargol solution decreased as impregnation proceeded. Later, Holmes (1943) found that while the pH value of a Protargol solution only fell from pH 8.2 to 8.0 during an impregnation period of 24 hr., if copper was present, the pH value fell to 6.6. Thus, there was some information available about the changes which occurred during impregnation, but their significance was unknown. In the present series of experiments an attempt was made to measure these various changes quantitatively, with the ultimate aim of analysing the impregnation process. At the same time

factors such as pH, temperature of impregnation and the amount of metallic copper in the solution were varied and correlated with the type of staining produced. Protargol-S and pre-1939 Bayer Protargol were used in the initial experiments and later other types of silver proteinates and protein-silver nitrate mixtures were employed to determine which properties of silver proteinates effected the production of a specific nerve stain.

The criteria used in deciding whether a given section was well stained were:

(a) The contrast, by either colour or intensity, between nerves and other tissues (Pl. 1, figs. 4-6).

(b) The absence of connective tissue staining, particularly reticulin (Pl. 1).

(c) The extent which peripheral, including autonomic nerves, were stained (Pl. 1, figs. 4-6).

(d) The extent to which the central nervous system and ganglia were stained (Pl. 1, figs. 1-3).

MATERIALS AND METHODS

The standard tissues used in these experiments were rat and mouse heads, taken from animals fixed by intra-arterial injection. 10% formol was used in the initial experiments, but since formol was not the ideal or most suitable fixative for all tissues, the results of these experiments were confirmed with heads fixed with Bouin's fixative and alcohol-formol-acetic (50% alcohol 90 ml.:formol 5 ml.:glacial acetic acid 5 ml.). The heads were decalcified by Bensley's decalcifier (equal volumes of 50% formic acid and 20% sodium acetate) for 72 hr., after which they were well washed in running tap water. Sections of rat brain and pieces of muscle which did not require decalcification were also used. It was appreciated that the method of dehydration and embedding could alter the stain, but since the mechanism of the staining process was under consideration and not the study of an individual structure, it was felt necessary to standardize the method for the preparation of sections, so that the tissues were dehydrated in a graded series of ethyl alcohol, cleared in benzene and embedded in 56° C. paraffin wax. It was found that although changes in procedure altered the extent to which various nervous elements were stained, they did not affect the overall staining of one section in relation to another section from the same block of tissue.

Bodian's method of impregnation (1936) was used as a standard technique and except in one experiment, where a physical developer was used to replace the hydroquinone-sodium sulphite developer, steps 2 to 6 in the following scheme were kept constant, although numerous variations were introduced during impregnation (step 1). The standard technique was as follows:

(1) After dewaxing and taking through graded alcohols to water, sections were impregnated for 16-24 hr. at 37° C. in a 1% solution of silver proteinate to which approximately 4 g. of clean copper wire (1.6 mm. in diameter) had been added per 100 ml. of solution.

(2) Sections were rinsed in distilled water and developed for 5 min. in a solution containing: hydroquinone, 1 g.; sodium sulphite (anhydrous), 10 g.; distilled water, 100 ml.; this developer contained double the quantity of sodium sulphite used by Bodian (Peters, 1955*b*).

(3) The developer was removed by washing the sections in running tap water for 10 min.

(4) Sections were taken through distilled water and immersed in 0.2% gold chloride, acidified with 1 drop of glacial acetic acid per 100 ml. of solution, for 10 min.

(5) The excess gold was removed by rinsing in distilled water and the sections transferred to 2% oxalic acid for 2 min. They were then washed in running tap water for 5 min. and the residual silver removed by immersing in 5% sodium thio-sulphate for 10 min.

(6) Finally, sections were washed in running tap water for 10 min., rinsed in distilled water, taken through graded alcohols to xylene and mounted in Canada Balsam.

The hydrogen ion concentrations in the impregnating solutions were measured with a Pye Universal pH meter, and the same instrument was employed as a milli-volt meter to measure the potential of the concentration cell used in determining the silver ion concentration in the proteinate solutions (0.1 M silver nitrate was used in the standard half cell and the activity coefficient of this solution was taken to be 0.72).

The silver deposited on to the copper wire during impregnation was determined as silver chloride, the silver being dissolved from the surface of the wire with 4% nitric acid. The protein was precipitated from the resulting solution with phosphotungstic acid and the silver then precipitated with sodium chloride. The silver chloride was filtered off after the solution had stood for 12 hr. and was then dried and weighed.

Copper from the wire went into solution during impregnation and after removal of protein from solution by phosphotungstic acid, this copper was determined by adding sodium diethyldithiocarbamate to the protein-free filtrate. The resulting golden-brown colour was checked visually against standard copper sulphate solutions. This method was not considered to be very accurate.

PROPERTIES OF SILVER PROTEINATES

The silver proteinates listed in Table 1 were used in the experiments. Of these eleven compounds, pre-1939 Bayer Protargol, Winthrop batches N-051 and N346BJ, Heyden batch 8214, Bayer Albargin and the American Pharmaceutical Company proteinate were relatively old samples that had been kept in the laboratory for some time. The other proteinates were obtained recently.

According to the classification of Martindale (1952) and Osol & Farrer (1955) these proteinates were classified as 'strong' (containing 7.5–8.5% silver) and 'mild' proteinates (19.0–23% silver). While most of the proteinates fitted into this classification, Albargin (15% silver) fell between the two groups. 1% solutions of the strong proteinates showed a greater degree of ionization than those of mild proteinates, and determinations with a silver electrode (Table 1) showed that 1% solutions of strong proteinates had silver concentrations of the order of 10^{-4} M, while for mild proteinates the value was between 10^{-6} and 10^{-8} M. The one exception was the proteinate manufactured by the American Pharmaceutical Company, for this sample,

which was old and dark in colour like the mild proteinates, gave a value of $6.7 \times 10^{-7} M$. When the concentration of the ionized silver was measured in solutions of Bayer Protargol, Protargol-S and the Heyden proteinate of different strength, it was found that the silver ion concentration was not proportional to the concentration of proteinate in solution. Ionization was suppressed as the concentration of proteinate increased, so that these compounds were behaving like weakly ionized salts, as would be expected of metallo-organic compounds.

As seen in Table 1, most of the strong proteinates were light or medium brown in colour, while the mild proteinates were in the form of shiny black granules. This probably gave some indication of the colloidal particle sizes of the compounds, so

Table 1. *Properties of silver proteinates*

Manufacturer or origin	Name or batch no.	Type	Silver content (%)	Description	Properties of 1% solution (mean values)	
					pH	Ionized silver concn. (M)
Bayer Products Ltd.	Protargol	Strong	About 8*	Light brown powder	8.0	7.5×10^{-4}
Winthrop-Stearns, Inc.	Protargol-S	Strong	7.5-8.5†	Light brown powder	8.3	7.1×10^{-4}
Winthrop Chemical Co. Inc.	Strong silver protein N-051	Strong	7.5-8.5‡	Light brown powder	8.1	9.6×10^{-4}
Winthrop Chemical Co. Inc.	Strong silver protein N346BJ	Strong	7.5-8.5‡	Light brown powder	8.1	9.0×10^{-4}
Boots Pure Drug Co. Ltd.	Argentoprot. B.P.	Strong	7.5-8.5§	Medium brown powder	9.1	1.4×10^{-4}
British Drug Houses Ltd.	Silver protein	Strong	About 8	Medium brown powder	9.1	1.4×10^{-4}
Heyden Chemical Corp.	8214 U.S.P.	Strong	7.5-8.5‡	Medium brown powder	7.6	3.2×10^{-4}
American Pharmaceutical Co. Inc.	Silver protein strong	Strong	7.5-8.5§	Black tablets	8.7	6.7×10^{-7}
Bayer Products Ltd.	Albargin	—	15	Shiny black granules	7.6	1.6×10^{-8}
British Drug Houses Ltd.	Silver protein	Mild	About 20	Shiny black granules	9.3	3.3×10^{-6}
Boots Pure Drug Co. Ltd.	Argentoprot. Mit. B.P.C.	Mild	19-23§	Shiny black granules	10.0	7.9×10^{-8}

References to silver contents

* Holmes (1943), Romanes (1950).

† Osol & Farrer (1955).

|| Information supplied by the manufacturer.

‡ Information supplied by Bayer Products Ltd.

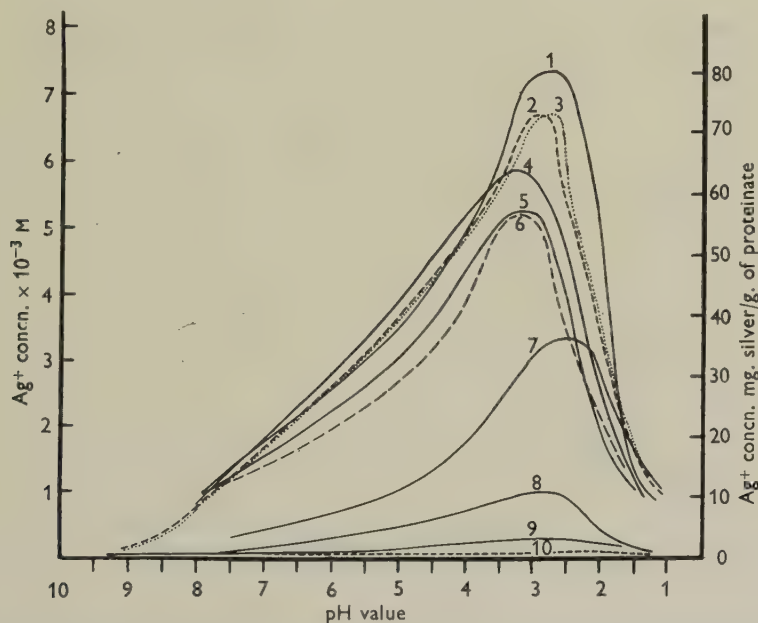
§ Martindale (1952).

that as pointed out by Romanes (1950), the lighter coloured compounds would have a smaller particle size than the other compounds. The small size of the colloidal particles in Protargol was emphasized by Neegaard (1923), who gave their size as $13\mu\mu$ and found that one-third of the silver was in the colloidal state and two-thirds in true solution in a 0.65% solution of Protargol.

Little information was available about the chemical composition of silver proteinates. Neegaard (1923) recorded that the original Protargol was an albumose-silver compound and this was supported by Romanes (1950), who stated that it was a combination of silver with partially hydrolysed egg albumen. Of the other proteinates, Davenport, Porter & Myhre (1952) reported that pharmaceutical peptone together with gelatin had been used by Winthrop-Stearns in the preparation of

Protargol, and gelatin was also used in the manufacture of Albargin, which was labelled as a gelatose-silver compound. Thus, the different preparations varied considerably in their compositions. Even different batches of the same product varied and the fact that only certain batches of Protargol were suitable for staining nerves was well known.

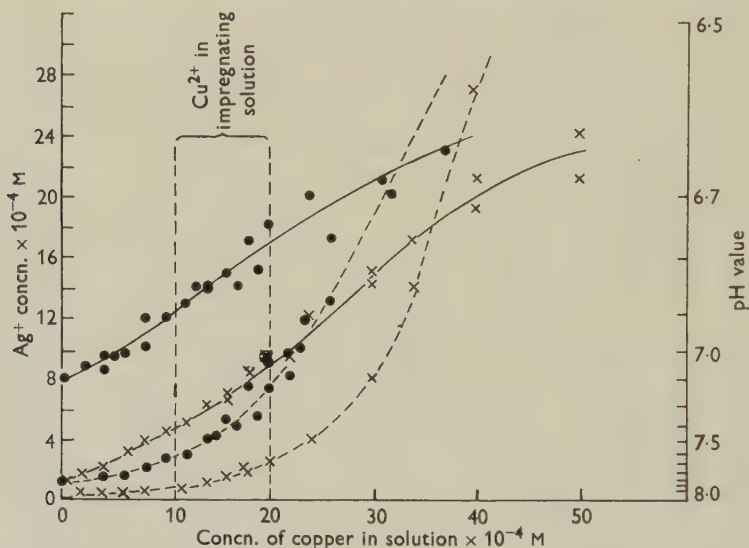
Mean values for the pH value of 1% solutions of different proteinates were as given in Table 1, although individual solutions varied by as much as ± 0.2 pH units. Furthermore, proteinate solutions were not stable, and at room temperature both the pH values and silver ion concentrations in the solutions changed slowly with time. These changes were accelerated by heat, so that at 37° C. the pH value of a Protargol solution fell from 8.3 to 7.6 in 24 hr.



Text-fig. 1. The silver ion concentration in 1% solutions of proteinates at different pH values. The pH values were attained by the addition of 1 M nitric acid and for the sake of clarity experimental points have been omitted from the graph. The numbers on the curves correspond to the following compounds. (1) Protargol-S; (2) Boots Argentoproteinium B.P. (strong); (3) Bayer Protargol; (4) British Drug Houses Strong Protein (8% Ag); (5) Winthrop batch N346BJ; (6) Winthrop batch N-051; (7) Heyden Chemical Corporation compound; (8) British Drug Houses Mild Protein (20% Ag); (9) Bayer Albargin; (10) Boots Argentoproteinium B.P.C. (Mild) and American Pharmaceutical Co. Proteinate. The scale on the right gives the weight of ionized silver in mg./g. of proteinate and shows that Protargol, Protargol-S and Boots strong proteinate are almost completely ionized at pH 3.

Holmes (1943) recorded that there was a marked decrease in the pH value of a copper-containing Protargol solution during impregnation. Clearly, such a change in the pH value may affect the degree of ionization of the proteinate and to determine whether or not this was the case, 1 M nitric acid was added drop by drop to 1% proteinate solutions, while the pH values and silver ion concentrations in the

solutions were measured. In all cases (Text-fig. 1) a decrease in the pH value was accompanied by an increase in the ionic silver concentration. A maximum value was reached at pH 2.5–3.5 (in the region of the isoelectric points of the proteins) and beyond this the silver ion concentration decreased. The addition of acid also produced a heavy precipitate on the alkaline side of the maximum silver concentration, but this began to dissolve again once the maximum was passed. In solutions of Protargol, Protargol-S and Boots strong Argentoproteinium, practically all of the silver was in the ionic state at pH 3 and, with the exception of the American Pharmaceutical Company compound, the strong silver proteinates were more highly ionized, at all pH values, than the mild proteinates. The importance of this observation became apparent when the staining properties of the compounds were considered.



Text-fig. 2. The addition of 0.1 M cupric nitrate to 50 ml. portions of 1 % Bayer Protargol and 1 % Boots Argentoproteinium B.P. (strong), showing the changes in silver ion concentration (full lines) and pH value (broken lines). ●—●, Bayer Protargol silver ion concentration; ●---●, Bayer Protargol pH value; ×—×, Boots Argentoproteinium silver ion concentration; ×---×, Boots Argentoproteinium pH value. The range of copper concentrations, determined at the end of impregnation, in solutions of Bayer Protargol containing 4 g. of copper per 100 ml. of solution is given (see Table 2).

Since Bodian (1936) found that copper went into solution during impregnation, the effect of adding 0.1 M cupric nitrate to 1 % solutions of proteinates was determined. In all cases this led to a decrease in the pH value, together with an increase in the silver ion concentration; the changes which occurred in 1 % solutions of Protargol and Boots Argentoproteinium are shown in Text-fig. 2. No doubt the addition of cupric nitrate resulted in a replacement, by copper, of some of the bound silver of the proteinate, so that the silver ion concentration increased. A combination between copper and the acidic groups of the protein could account for the decrease in the pH value.

IMPREGNATION IN PROTARGOL AND PROTARGOL-S

In the following experiments, impregnating solutions were removed from the incubator at intervals during impregnation and the pH values and hydrogen ion concentrations measured.

(a) *The role of copper and the effect of temperature and proteinate concentration on staining*

Both Protargol and Protargol-S gave good staining when sections were impregnated at 37° C. by the modified Bodian technique, although Protargol-S gave the better overall staining of nervous elements and especially of the autonomic

Table 2. *Impregnation in 1% solutions of Bayer Protargol, showing the changes in pH value, silver concentration and copper concentration*

2	3	4	5	6	7	8		9	10	11
Vol. of soln. (ml.)	Temp. of im- pregn. (° C.)	Dura- tion of im- pregn. (hr.)	pH change	Wt. of copper wire (g.)	Concn. of* ionic silver released by pH change (M)	Silver plated on to copper wire		Concn. of copper in solution (M)	Ag/Cu (8(b)/9)	Ag ⁺ released by pH change Ag on wire (7/8(b))
						(a) (mg./g. wire)	(b) Molarity as solution (M)			
100	18	15.75	7.9→7.3	4.1	1.5×10^{-3}	6.8	2.6×10^{-3}	0.96×10^{-3}	2.7	0.58
50	37	16	8.1→6.1	1.9	2.8×10^{-3}	10.0	3.5×10^{-3}	1.2×10^{-3}	2.9	0.80
100	37	15.75	7.9→6.3	4.4	2.8×10^{-3}	11.0	3.8×10^{-3}	1.0×10^{-3}	3.8	0.74
50	37	47	8.1→6.3	2.4	2.9×10^{-3}	9.8	4.3×10^{-3}	2.3×10^{-3}	1.9	0.69
50	56	50	7.9→6.0	2.1	2.9×10^{-3}	11.0	4.2×10^{-3}	1.9×10^{-3}	2.2	0.69
100	56	24	8.2→6.2	4.7	2.9×10^{-3}	9.1	4.0×10^{-3}	1.5×10^{-3}	2.7	0.72
50	56	47	8.1→5.9	2.4	3.0×10^{-3}	10.0	4.3×10^{-3}	1.9×10^{-3}	2.2	0.70
100	37	11.5	8.1→6.6	12.0	2.1×10^{-3}	6.8	4.1×10^{-3}	2.7×10^{-3}	1.5	0.51
100	37	11.5	8.1→6.75	8.0	1.8×10^{-3}	9.2	3.7×10^{-3}	1.4×10^{-3}	2.6	0.48
100	37	11.5	8.1→7.2	4.0	1.4×10^{-3}	11.4	2.2×10^{-3}	0.45×10^{-3}	2.3	0.64
100	37	11.5	8.1→7.7	0	0.8×10^{-3}	—	—	—	—	—

* The value for the concentration of ionic silver released by the pH change was determined on the basis of Text-fig. 1.

system. During impregnation by the standard method with Protargol, the pH value and silver ion concentration in the impregnation solution both decreased with time as shown in Text-fig. 3. In this particular case two slides of sections were in the impregnating solution, but it was shown subsequently that the presence or absence of sections had no effect on the changes in the solution. The initial fall in the pH value and silver ion concentration was rapid, but the rate of fall of these decreased with time; similar results were obtained with Protargol-S. Visible changes were also observed during impregnation, in that silver plated-out on to the copper wire, which simultaneously became coated with a protein precipitate, while at the same time the brown colour of the impregnating solution assumed a green tint due to copper going into solution from the copper wire. About 10 mg. of silver/g. of copper wire were plated-out during impregnation and the final concentration of copper in the solution was about $1-2 \times 10^{-3}$ M; some values are given in Table 2.

The effects of temperature of impregnation, proteinate concentration and the amount of copper wire in the solution were as shown in Table 3, which gives examples of the types of results obtained in the experiments. In recording the values of pH and silver ion concentration during impregnation, it was found that the rate of fall of these values was accelerated when either the amount of copper added to the

solution or the temperature of impregnation was increased. Thus, the concentration of ionic silver and the pH value of the solution were less, at any given time, in a solution containing 12 g. of copper wire per 100 ml. than in a solution containing only 4 g. of copper. Such changes were also related to the type of staining obtained, for an increase in either the amount of copper or the temperature of impregnation led to a decrease in the overall intensity of staining together with an increased contrast between nerve fibres and other tissues.

The correlation between final staining intensity and the concentration of silver ions in the solution was also confirmed by experiments in which the proteinate concentration was varied (Table 3), while the amount of copper in the solution and the temperature of impregnation were kept constant.

Table 3. *The effect of temperature, proteinate concentration and copper on impregnation*

Period of im- pregn. (hr.)	Strength of solu- tion (%)	Temp. of im- pregn. (° C.)	Copper wire in solution	pH value		Silver concentration		Staining results
				Initial	Final	Initial (M)	Final (M)	
(a) Effect of temperature (Bayer Protargol)								
24	1	18	4 g./100 ml.	8.1	7.55	7×10^{-4}	5×10^{-4}	Increase in contrast between nerve fibres and other tissues as temperature was raised. Little contrast at 18° C. Non-nervous tissues only faintly visible at 56° C. Order of intensity of general staining: 18 > 37 > 56° C. Best result at 37° C.
	1	37	4 g./100 ml.	8.1	6.85	7×10^{-4}	0.8×10^{-4}	
	1	56	4 g./100 ml.	8.1	6.40	7×10^{-4}	0.4×10^{-4}	
(b) Effect of proteinate concentration (Protargol-S)								
24	1	37	4 g./100 ml.	8.2	6.80	7×10^{-4}	1×10^{-4}	Decrease in intensity of staining of all tissues with decrease in concentration of proteinate. In 0.25 % solution, staining very light
	0.5	37	4 g./100 ml.	8.2	6.95	3.5×10^{-4}	0.9×10^{-4}	
	0.25	37	4 g./100 ml.	8.2	7.05	2.5×10^{-4}	0.2×10^{-4}	
(c) Effect of amount of copper (Bayer Protargol) (see Table 2)								
11½	1	37	0 g./100 ml.	8.1	7.70	8.5×10^{-4}	4.0×10^{-4}	Increase in contrast between nerve fibres and other tissues as amount of copper in solution increased. With no copper nerves difficult to distinguish against equally intense background. Best results with 4 g. of copper. With 12 g. of copper, nerve staining patches and pale with muscle almost grey in colour
	1	37	4 g./100 ml.	8.1	7.20	8.5×10^{-4}	2.0×10^{-4}	
	1	37	8 g./100 ml.	8.1	6.75	8.5×10^{-4}	0.8×10^{-4}	
	1	37	12 g./100 ml.	8.1	6.60	8.5×10^{-4}	0.3×10^{-4}	

The initial pH value of the impregnating solution also played an important part in determining the type of staining produced. When the initial pH values of 1 % solutions of Protargol and Protargol-S, containing 4 g. of copper per 100 ml. of solution, were changed by adding boric acid-borax buffers and the sections impregnated at 37° C.; the best results were obtained at pH 8.3. At pH 8.7 there was little contrast between nerves and other tissues and at pH 7.6 and 7.8 the staining was coarse and more connective tissue was visible than at higher pH values. As would be expected, during impregnation in a buffered solution there was little change in the pH value, but when the pH value was adjusted with ammonia there was a

decrease, so that, for example, when the initial pH value was 9.3 it fell to 7.6 during 16 hr. Since the nerve staining in the latter solution was better than that in a solution buffered to pH 9.3, it appeared that the fall in pH during impregnation played an important role in the impregnation process.

The function of the metallic copper in the impregnating solution could not be simulated by adding a copper salt to the solution. Sections impregnated in a 1% solution of Protargol containing approximately the same concentration of copper ($1 \times 10^{-3}M$ cupric nitrate) as that found in a solution at the end of impregnation when metallic copper was present (Table 2), showed a similar type of staining to a copper-free solution. This suggested that copper ions themselves played little part in determining the type of staining produced by a solution, and the main function of the metallic copper appeared to be the removal of silver ions from the impregnating solutions. This point will be referred to later.

(b) *The effect of time on staining*

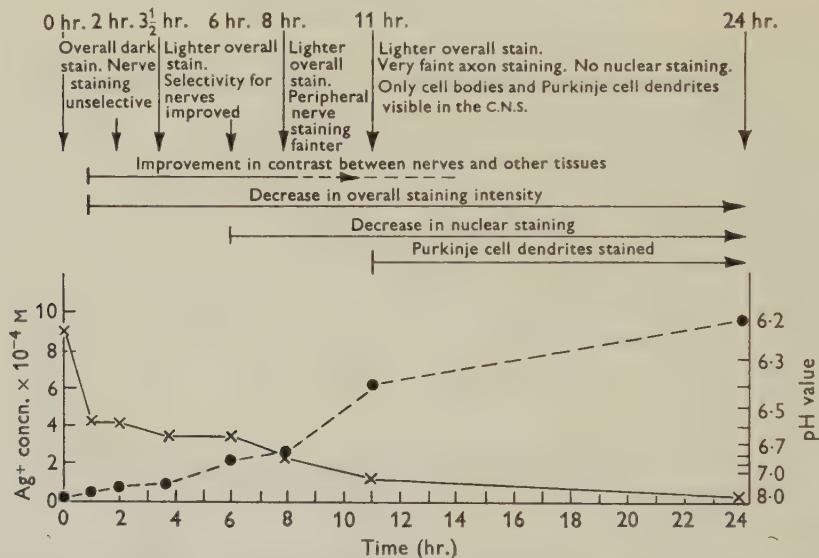
Peters (1955a) showed that during impregnation two different reactions took place between the sections and silver ions. Some of the silver combined with the sections and remained in the unreduced state, reducible silver, while a smaller fraction was reduced to form silver nuclei, which during development in hydroquinone-sulphite acted as centres for the reduction and deposition of the reducible silver. The formation of silver nuclei appeared to be irreversible and the relative amount of silver in the form of nuclei was determined by immersing sections in sodium sulphite, which removed the reducible silver, and then developing in a glycine physical developer for a constant period of time. Under these conditions the intensity of the stain was dependent on the concentration of silver nuclei in the sections (Peters, 1955a, c).

A comparison of the amount of reducible silver plus silver nuclei in different sections was indicated by the intensity of the stain obtained after development in the hydroquinone-sodium sulphite. Thus, since the formation of silver nuclei was irreversible, when sections were put into an impregnating solution simultaneously, removed after different lengths of time and developed in hydroquinone-sodium sulphite, any decrease in the intensity of staining associated with a longer impregnation was due to a decrease in the reducible silver fraction in the section.

When sections were impregnated at either 37 or 56° C. in copper-containing Protargol-S solutions, and removed at intervals over a period of 24 hr., hydroquinone-sodium sulphite development showed that after the first hour of impregnation, the amount of reducible silver in combination with the section decreased with time. Conversely, there was an increase in the amount of silver in the form of nuclei.

In a further experiment, sections of rat head were impregnated at 56° C. in solutions of 1% Protargol containing 4 g. of copper per 100 ml. of solution. One set of sections was impregnated in sequence, i.e. only one section was in the solution at any given time and was removed from the solution and replaced by another, at intervals over 24 hr. A second set of sections, which acted as controls, was put into the solution at the beginning of impregnation and one section was removed at the same time as each section of the first set. The results of this experiment were shown

in Text-fig. 3. Sections were removed from the solution at times shown by each vertical arrow in Text-fig. 3, and the results obtained by comparing the changing nature of the stain in the sections impregnated in sequence are shown between the vertical arrows. The horizontal arrows refer to the control sections and indicate trends which were visible in these sections impregnated from zero time. This experiment showed that after the first hour of incubation there was a decrease in the overall staining intensity and since reducible silver was removed most readily from the background elements, the contrast between the nerve fibres and other tissues improved as impregnation continued, so that the stain was self differentiating. A decrease in nuclear staining occurred after the 6th hour of incubation and there was almost no further axon staining in the second half of the period of impregnation, when the only new nervous elements to stain were the Purkinje dendrites. The following examples illustrate these points.



Text-fig. 3. The effect of time on staining formol fixed sections of rat head impregnated at 56° C. in 100 ml. of 1 % Bayer Protargol containing 4 g. of copper wire. The graph shows the changes in silver ion concentration (full line) and pH value (broken line). Above the graph, the horizontal arrows indicate continuous trends in the staining of sections impregnated from zero time, while the vertical lines indicate the removal of sections from the impregnating solution. The intervals between the vertical arrows indicate the times of impregnation of individual sections stained in sequence, and the type of staining produced during these times is indicated between the lines. See text for full explanation.

(1) A section put in at zero time and incubated for 24 hr. showed a selective stain for axons, cell nuclei and Purkinje cell dendrites. In a section impregnated from the 11th to the 24th hour, the only nervous elements to be well stained were the Purkinje cell dendrites; the overall staining was light and there was almost no staining of axons and cell nuclei. Thus, the only feature shared by these two sections was the staining of Purkinje cell dendrites, and as can be seen from Text-fig. 3 these only stained in sections removed from the solution after the 11th hour.

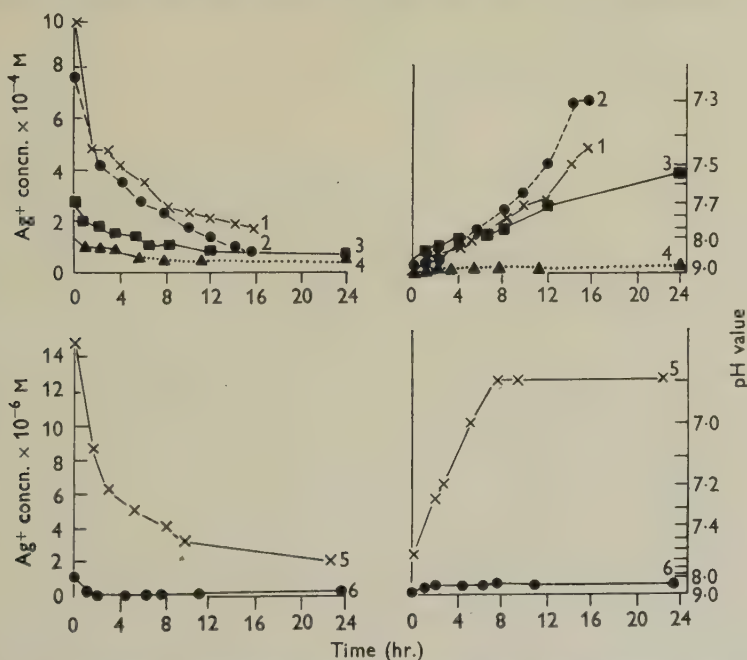
(2) A section put in at 8 hr. and removed at 11 hr. was much lighter in overall staining intensity, including axons and cell nuclei, which were only faintly visible, than a section put in at $3\frac{1}{2}$ hr. and removed at 6 hr.

Under the same conditions, Protargol-S showed a slightly different sequence of staining, for satisfactory nerve staining persisted over the 24 hr., although the intensity decreased and there was some staining of neuro-keratin in the later stages. Purkinje cell dendrites were never stained well in Protargol-S solutions (Pl. 1, fig. 1).

The correlation between the trends in staining and the changes in pH value and silver ion concentration during impregnation will be discussed later.

IMPREGNATION IN OTHER SILVER PROTEINATE SOLUTIONS

As a basic test of their staining potentials, solutions of the other silver proteinates, listed in Table 1, were used to replace Protargol in the modified Bodian technique. In addition to the initial pH values, temperatures of impregnation and the concentrations of the solutions were varied. The only compounds which produced staining like that obtained with Protargol and Protargol-S were the Winthrop compounds batches N-051 and N346BJ, but these gave a somewhat lighter and less specific stain. In the case of the Boots and British Drug Houses strong silver proteinates the stain was granular and showed little specificity for nerves, even when



Text-fig. 4. A comparison of silver ion concentration and pH value changes, with time, in 1% solutions of proteinates containing 4 g. of copper per 100 ml. of solution at 37°C . The graphs on the left show the changes in silver ion concentration and those on the right the corresponding changes in pH value. The number on the curves refer to the following proteinates: (1) Winthrop Batch N346BJ; (2) Bayer Protargol; (3) Heyden Chemical Corporation strong proteinate; (4) Boots Argentoproteinum B.P. (strong); (5) Bayer Albargin; and (6) American Pharmaceutical Co. strong proteinate.

the initial pH value of the solutions was adjusted to pH 8.1–8.3 with acid. Both of the mild proteinates, together with Albargin, produced a light stain which was not specific for nerves. The obvious reason for this light stain was the low concentration of ionic silver present in the solutions (Table 1).

Interesting results were obtained with the Heyden strong silver proteinate. Although a normal solution of this gave a light stain which showed little specificity for nerves, the type of staining was completely transformed when the initial pH value of the solution was changed from 7.6 to 8.2–8.4 by the addition of ammonia, for the results were then as good as those produced by Protargol (Pl. 1, fig. 2). From this it appeared that the initial pH value of the normal solution was too low, for the staining produced by such a solution was similar to that obtained if the pH value of a Protargol solution was changed to pH 7.6 by the addition of acid.

The changes in pH value and silver ion concentration that took place when sections were impregnated in 1% copper-containing solutions of different proteinates were compared directly (Text-fig. 4). It was clear that in all solutions similar changes in pH value and silver ion concentration took place during impregnation. The importance of the initial pH value of the solution was emphasized by the experiments with Heyden compound, but since no such great change in the specificity of the stain was obtained when the initial pH values of the Boots and British Drug Houses strong proteinates were adjusted to pH 8.3, it seemed likely that the protein used in the preparation of the proteinate also played an important part in determining the staining properties of the preparations. This point was investigated by the use of various protein-silver nitrate mixtures.

IMPREGNATION IN PROTEIN-SILVER NITRATE MIXTURES

Solutions of 2, 1 and 0.5% of dried egg albumen, blood albumen, casein, gelatin and bacteriological peptone (all obtained from British Drug Houses Ltd.); 2% fresh citrated human blood plasma and 3.5% fresh egg albumen were used as the protein fraction in these experiments. Enough 2% silver nitrate was added to the protein solution to give a silver ion concentration of $2.0\text{--}5.0 \times 10^{-4}\text{M}$ when the pH value of the solution was adjusted to pH 8.2–8.5 by the addition of ammonia, sodium hydroxide or 0.1M boric acid-borax buffer. The volume of silver nitrate necessary to produce this silver ion concentration varied with the protein. Sections were impregnated for 16 hr. at 37° C. in the absence of copper, developed in the hydroquinone-sodium sulphite developer and gold toned.

The solutions containing either gelatin, blood albumen, fresh citrated blood plasma or bacteriological peptone produced some nerve staining, but the results were poor and relatively unspecific for nerves. On the other hand, dried egg albumen-silver nitrate mixtures gave an excellent stain which was specific for nerves (Pl. 1, fig. 3). Almost no connective tissue was visible and the results were as good as those produced by Protargol (Pl. 1, fig. 4) and Protargol-S (Pl. 1, figs. 1, 5 and 6). Fresh egg albumen gave inconsistent results, although the staining was good in some cases. Casein-silver nitrate mixtures also stained nerve selectively, but the results differed from those produced by the dried egg albumen mixtures in that the stain was rather coarse and somewhat uneven, particularly in muscle. The addition of

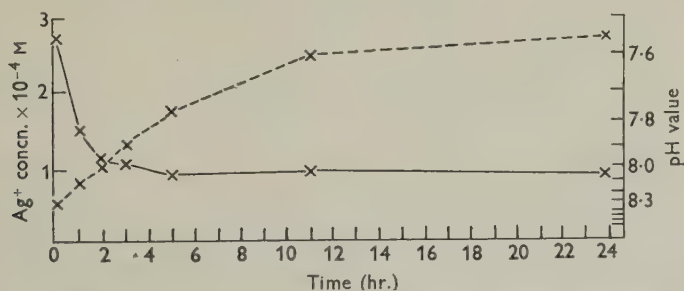
metallic copper to either the dried egg albumen or casein mixtures had little effect on the result beyond promoting some connective tissue staining. It was clear from these experiments that the protein fraction of the proteinate did play a part in determining the staining properties of the solution.

Table 4. *Impregnation in egg albumen-silver nitrate mixtures of different compositions and at different temperatures*

Composition of impregnating solution: 50 ml. of 0.5 % dried egg albumen, x ml. of 2 % silver nitrate; pH value adjusted with ammonia

Solution	Control						
	1	2	3	4	5	6	7
(1) Properties of impregnating solution							
(a) Silver nitrate added (ml.)	1.8	1.8	1.8	1.8	1.8	1.4	2.2
(b) Initial pH value	8.3	7.7	9.0	8.3	8.3	8.3	8.3
(c) Initial silver ion concentration ($\times 10^{-4}$ M)	2.3	7.7	0.4	2.3	2.3	1.2	6.3
(d) Temperature of impregnation ($^{\circ}$ C.)	37	37	37	18	56	37	37
(e) Final silver ion concentration ($\times 10^{-4}$ M)	0.6	0.4	0.1	1.2	0.3	0.2	0.3
(f) Final pH value	7.7	6.7	8.3	8.1	7.8	7.7	7.8
(2) Staining results							
(a) Staining intensity							
Overall	+++	++	+++	++	++	+	++++
CNS	+++	++	+++	+++	++	+	++++
Connective tissue	+	+++	+	++	+	+	++
(b) Selectivity of nerve staining							
In muscle	000	0	00	00	000	0	00
In CNS	000	00	00	00	000	0	000
In subepithelial tissue	000	00	00	0	000	0	00

Key to symbols. Staining intensity: + faint; through ++ and +++, to ++++ intense. Selectivity of nerve staining: 0 very poor; 00 poor; 000 good.



Text-fig. 5. The change in the pH value (broken line) and silver ion concentration (full line) during impregnation in a solution containing 50 ml. of 0.5 % dried egg albumen and 1.8 ml. of 2 % silver nitrate. The initial pH value was adjusted to pH 8.3 by the addition of ammonia. Note the similarity between the shapes of these curves and those obtained with silver proteinates.

The best results were obtained with dried egg albumen-silver nitrate mixtures when sections were impregnated at 37° C. in a solution consisting of 50 ml. of 0.5 % dried egg albumen solution with 1.8 ml. of 2 % silver nitrate, the pH value of which was adjusted to pH 8.3 with ammonia (Pl. 1, fig. 3). A typical example of the

changes occurring in the pH value and silver ion concentration during the course of impregnation in such a solution is shown in Text-fig. 5, and it will be seen that these changes were very similar to those which took place in copper-containing Protargol solutions (Text-fig. 3). Full details of the staining procedure employing dried egg albumen-silver nitrate solutions have been given elsewhere (Peters, 1958).

The egg albumen-silver nitrate mixtures proved to be very useful experimentally, since the different factors involved in impregnation could be easily studied by altering the composition of the mixtures. The composition, properties and the staining results obtained in such experimental solutions are given in Table 4, in which it will be seen that:

(1) In impregnating solutions at the same temperature and initial pH value (solutions 1, 6 and 7), the intensity of staining depended upon the silver ion concentration.

(2) The intensity of the stain and the specificity for nerves were influenced by the pH value of the impregnating solution (solutions 1-3), the best result being obtained at an initial pH value of 8.3 (solution 1).

(3) Good results were obtained when sections were impregnated at pH 8.3 at either 37° C. (solution 1), or 56° C. (solution 5). At 18° C. (solution 4) the results were patchy and coarse.

Similar results were obtained with casein-silver nitrate mixtures.

DISCUSSION

From these experiments it was possible to form a picture of some of the chemical and physical processes that took place during normal impregnation with Protargol and Protargol-S. Due to the complexity of the system it was obviously necessary to simplify the reactions, but nevertheless the following formed an adequate working hypothesis. As impregnation proceeded, silver was lost from the impregnating solution (Text-figs. 3, 4) and this appeared to be effected mainly by a plating-out of silver on to the copper wire. Silver was also taken from the solution by the section, but that this was insignificant was shown in previous experiments (Peters, 1955*c*), where a 15 μ section 1 cm.² contained only about 2×10^{-5} g. of silver compared to the amount of silver plated out on to the copper wire (approximately 1×10^{-2} g./g. of copper wire).

On the basis of their chemical equivalents, for every two atoms of silver plated out one copper ion would enter solution. Some values for the concentration of copper going into solution, together with the corresponding concentration of silver plated-out, were given in Table 2, but it was found that in almost every case the value of the ratio Ag/Cu was more than 2; the mean value being about 2.5. Nevertheless, the results were in some agreement with the theoretical considerations and some part of the discrepancies were attributable to (a) the protein which precipitated on to the surface of the copper during impregnation; this could not be removed, so that it was included in the figures for the amount of silver plated-out; and (b) the method for determining the copper in solution was not considered to be very accurate, while copper bound with the proteinate could not be taken into account.

The loss of ionic silver was accompanied by a decrease in the pH value of the

solution and these changes appeared to be related, for both were accelerated by either heat or an increase in the amount of copper wire added to the solution (Table 3). Since the addition of a copper salt to a Protargol solution also decreased the pH value of that solution, it was concluded that the decrease in the pH value of a copper-containing solution was the resultant, primarily, of the decrease resulting from copper going into solution (Text-fig. 1), and secondarily, of the decrease due to the instability of the Protargol solution at the temperature of impregnation.

On the basis of Text-fig. 1 a calculation was made of the amount of ionic silver that would be released from the Protargol as a result of the decrease in pH value during impregnation. The results (column 7, Table 2) showed that this accounted for only 50–80% of the silver plated-out on to the copper (column 11, Table 2). Thus, it appeared that there was no simple correlation between the release of ionic silver and the fall in pH value. It was impossible to take into account the displacement of silver from the proteinate by copper ions, and in the impregnating system the proteinate would probably release more than the calculated amount of silver to counteract the continual withdrawal of silver by the plating-out process.

These considerations, together with the results of staining sections in different solutions, suggested the following theory of chemical reactions taking place during impregnation. In the impregnating solution there was an equilibrium system between the solution, the reducible silver in the section and the colloidal fraction of the proteinate. Silver ions were removed from this system by (*a*) plating-out on to the copper, and (*b*) the formation of silver nuclei in the section. It was found that the amount of silver removed by both sites increased as impregnation proceeded. To counteract this loss, the system tended towards equilibrium again by the withdrawal of silver ions both from the proteinate, which may be regarded as the reservoir for silver, and the reducible silver fraction of the section. The withdrawal from the reducible silver fraction accounted for the observed decrease in the overall staining intensity of sections as impregnation continued (Text-fig. 3), and this loss of silver appeared to take place most readily from the non-nervous elements in the section, as suggested by Glassner, Breslau & Agress (1954) to account for the improvement in contrast between nervous and non-nervous elements when sections were impregnated for long periods of time (Text-fig. 3). Such a theory would also explain the decrease in overall staining intensity, together with the improvement in differentiation at 56° C. as opposed to 37° C., for as the temperature of impregnation was increased the loss of ionic silver from the impregnating solution was accelerated (Table 3).

Clearly, the metallic copper took part in the impregnation process by removing silver ions from the solution. Its presence led to an improvement in the differentiation of the stain, but whether the copper ions released in exchange for the silver played any part in staining could not be determined, although it was found that in the absence of the metal, the presence of copper ions had no effect on the result, beyond tending to make the connective tissue stain. Copper ions would certainly enter into competition with silver ions for the binding sites in the proteinate and as Bodian (1936) showed, during impregnation, copper was deposited, together with silver, in the nerve fibres.

From the experiments, it was shown that the pH value, the silver ion concentra-

tion in the impregnating solution and the protein used in the formation of the proteinate were all important factors in determining the staining properties of a compound. Thus, only those solutions of proteinates and protein silver nitrate mixtures which initially contained more than about $1.3 \times 10^{-4}M$ ionic silver gave a sufficiently intense stain. The best results were produced when the initial pH value of the system was between 8.0 and 8.4, and the importance of this was demonstrated by the Heyden proteinate which gave excellent nerve staining when the initial pH value was raised from pH 7.6 to 8.3. Further, the changes in pH value that occurred during impregnation also appeared to play an essential part in staining. During impregnation by Bodian's method, sections were subjected to a continually falling range of pH values, and the type of staining obtained at different phases of the process was shown in the experiments where sections were stained in sequence (Text-fig. 3). Optimum conditions for nerves obtained at an early phase of impregnation and they were only lightly stained during the final stages. Changing the initial pH value of a solution showed that connective tissue stained most readily at low pH values (see Table 4), but in normal impregnation in Protargol, such values were only attained at a late stage when the silver ion concentration was also at a low level. Consequently, connective tissue was only lightly stained, if at all, by the Bodian and egg albumen-silver nitrate methods.

With the exception of the Heyden proteinate, other proteinates which had a sufficiently high silver ion concentration, i.e. the Boots and British Drug Houses strong Proteinates, still did not produce a good nerve stain even when the initial pH value of the solution was changed to pH 8.3. It seemed that this lack of nerve staining could be attributed to the protein fraction in these proteinates, for it was shown by the use of protein-silver nitrate mixtures that the protein played a definite part in determining the type of staining produced. Only the mixtures containing dried egg albumen or casein gave good results, and this confirmed the results of Romanes (1950), who obtained somewhat similar results when he impregnated sections in solutions produced by the addition of silver nitrate to hydrolysates of egg albumen, casein and gelatin; the former two hydrolysates produced good staining which was never obtained with the gelatin hydrolysate. It was also interesting that egg albumen appeared to have been used in the manufacture of Protargol (Neegaard, 1923; Holmes, 1943; and Romanes, 1950). While this indicated the importance of the protein, Porter & Davenport (1951) concluded, as a result of the study of a series of silver proteinates prepared from split proteins, that the manner in which a protein was degraded was more important in determining the selectivity of the stain than was the source of the protein itself.

Thus, it has been shown that the staining properties of a silver proteinate depended upon the pH value and silver ion concentration of its solution, as well as the protein used in the manufacture of the proteinate. The type of staining could also be varied by altering the amount of metallic copper added to the solution and the temperature of impregnation, but even then, the method of fixation, dehydration and embedding affected the result. The most satisfactory method of staining which emerged from this study was that in which silver nitrate was added to solutions of either dried egg albumen or casein, for in such mixtures the pH value and silver ion concentration could be controlled in a way that was not possible with silver proteinates.

SUMMARY

During impregnation, by Bodian's method, in solutions of Protargol and Protargol-S, there was an equilibrium between the silver combined with the section, the silver in the silver proteinate and the silver ions in solution. Silver ions were removed from the solution by (a) combination with the section to form reducible silver and silver nuclei, and (b) plating-out of silver on to the metallic copper; compared to the amount of silver plated-out on to the copper (about 1×10^{-2} g./g. of copper wire, 1.6 mm. diameter) the silver combined with the section (about $1-3 \times 10^{-5}$ g. in a 15μ section 1 cm.²) was negligible. This removal of silver led to a decrease in the silver ion concentration in the solution and, as a result, the equilibrium system was readjusted by the withdrawal of silver from the proteinate and the reducible silver fraction of the section. As silver plated-out, copper ions went into solution, so that at the end of impregnation, the concentration of copper in the solution was $1-2 \times 10^{-3}$ M. In part, the copper ions were responsible for the observed fall in the pH value of the solution during impregnation, but there was no evidence that the copper ions played any part in the actual impregnation process in the section itself. An increase in the temperature of impregnation or in the amount of metallic copper added to the solution, led to an increased rate of plating-out of silver and hence an increase in the rate of fall of both the silver ion concentration and pH value.

The conditions necessary to produce a specific stain for nerves were investigated using eleven different silver proteinates and various protein-silver nitrate mixtures. It was found that the initial pH value of the solution must be between 7.8-8.4, and that for the stain to be sufficiently intense, the initial silver ion concentration should be greater than 1.3×10^{-4} M. The protein in the silver proteinate played a part in determining the specificity of the stain for nerves.

This work was carried out during the tenure of a Post-graduate Fellowship in the University of Edinburgh. I wish to express sincere thanks to Prof. G. J. Romanes for his constant interest and advice during the course of this work, to Mr H. Tully for his able technical assistance and to Dr J. W. Minnis of the Biochemistry Department for carrying out silver chloride determinations on my behalf. Bayer Products Ltd. gave the Protargol-S and Boots Pure Drug Co. Ltd. gave samples of Argentoprotein; I am grateful to both of them for their co-operation.

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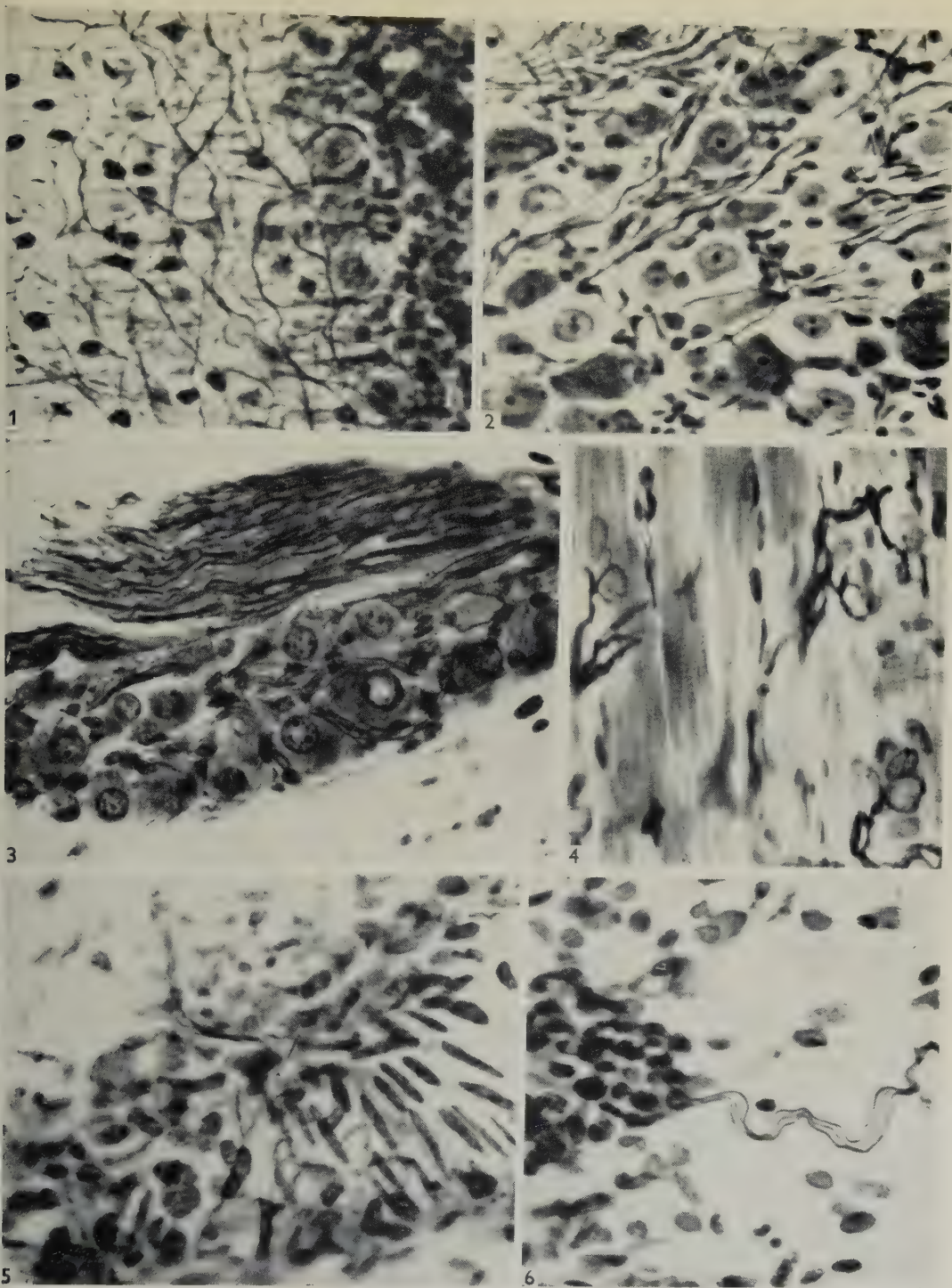
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EXPLANATION OF PLATE

All photomicrographs were taken from rat and mouse heads fixed by intra-arterial injection and decalcified in Bensley's decalcifier. After impregnation, sections were developed in hydroquinone-sodium sulphite and gold toned.

- Fig. 1. Formol fixed rat cerebellum. Section impregnated for 16 hr. at 37° C. in 1 % Protargol-S containing 4 g. of copper wire per 100 ml. of solution ($\times 510$).
- Fig. 2. Trigeminal ganglion from mouse fixed with formol. Section impregnated for 16 hr. at 37° C. in a 1 % solution of Heyden proteinate adjusted to an initial pH value of 8.3 with sodium hydroxide. The solution contained 4 g. of copper wire per 100 ml. ($\times 470$).
- Fig. 3. Autonomic ganglion from the tongue of a mouse fixed with alcohol-formol-acetic. Section impregnated for 16 hr. at 37° C. in a solution containing 100 ml. of dried egg albumen and 3.6 ml. of 2 % silver nitrate, the initial pH value of which was adjusted to 8.3 with ammonia ($\times 500$).
- Fig. 4. End plates from the head of a rat fixed in formol. Section impregnated for 16 hr. at 37° C. in 1 % pre-1939 Bayer Protargol containing 4 g. of copper wire per 100 ml. ($\times 350$).
- Fig. 5. Arteriole from the head of a mouse fixed in Bouin's fixative. The section was impregnated for 16 hr. at 37° C. in a 1 % solution of Protargol-S containing 4 g. of copper wire per 100 ml. Nerve fibres can be seen in association with the vessel.
- Fig. 6. Nerve fibres in the epiglottis of a mouse fixed in Bouin's fixative. The section was impregnated for 16 hr. at 37° C. in a 1 % solution of Protargol-S containing 4 g. of copper per 100 ml. ($\times 600$).



PETERS—STAINING OF NERVOUS TISSUE WITH SILVER PROTEINATES

(Facing p. 194)

AN EFFECT OF PYRONIN UPON THE RATE OF MATURATION OF INJURED PERIPHERAL NERVE FIBRES

By H. J. GAMBLE AND B. D. JHA

St Mary's Hospital Medical School

INTRODUCTION

Various drugs, hormones, vitamins, etc., have been employed in attempts to accelerate the processes of regeneration and maturation in injured peripheral nerves (for a summary, see Guth, 1956). Most of these attempts have been unsuccessful, but Bammer & Martini (1953) have claimed that sensory re-innervation of the rabbit's cornea is accelerated by the administration of polysaccharide pyrogens; they believed that irritation of the nerve cells and stimulation of their metabolic processes might be the mechanism involved.

Hoffman (1952) has reported that the outgrowth of buds from intact axons and their subsequent re-innervation of partially denervated muscle was accelerated in rats by the administration of 0.1 % pyronin B or G, or a combination of both these dyes in the animals' drinking water. His investigation was prompted, apparently, by the claim that pyronin is mutagenic in *Drosophila* and accelerates the rate of growth of these insects, possibly by attaching itself to and acting upon ribose nucleic acid bodies. He thought that a similar mechanism involving Nissl bodies in appropriate nerve cells might explain the accelerated axonal budding (and, by inference, increased rate of axoplasmic synthesis) which he observed.

It has been suggested (e.g. by Bodian, 1947) that axon regeneration is merely an artificially accelerated normal function of nerve cells. If this were so, and if Hoffman's tentative explanation of his own results is valid, then pyronin might be expected also to accelerate the processes of axonal regeneration. Experiments made to test this possibility are reported here.

MATERIALS AND METHODS

(1) Preliminary experiments were made upon the ventral caudal nerve in eleven young adult (180–200 g.) albino rats of either sex. The nerve was crushed with smooth-bladed watch-maker's forceps and allowed to regenerate for periods ranging between 14 and 21 days. Two of these animals were given 0.1 % pyronin G to drink and were killed after 14 days. The remainder were given tap water to drink and were killed after 15 days (two specimens), 16 days, 17 days, 19 days (two specimens), and 21 days (three specimens). The crushed portion of the nerve was identified and the centimetre distal to it was removed for fixation (lightly stretched upon a card) for 24–48 hr. in 1 % osmium tetroxide. The nerve was then teased and mounted in glycerine.

(2) The sural nerve was crushed unilaterally at a constant level in the leg in

seventeen young adult (180–200 g.) rats of either sex. The animals were then kept at room temperature for 30, 50 and 70 days. Seven animals were given 0.1 % solution of pyronin G in tap water to drink, the remaining twelve receiving tap water. In all other respects both groups were treated alike.

After the appropriate survival period, the crush level was identified under ether anaesthesia and a measured length of nerve (1 cm. distal to the crush) removed. This was lightly stretched upon a card for fixation in Flemming's fluid for 24–48 hr. Transverse sections (cut at 5μ after paraffin embedding) were stained by the Gutmann & Sanders (1943) technique for myelin and negative prints were made by direct projection on to bromide paper at $750\times$ diameter magnification. Counts and measurements of the myelinated fibres present were made and from these measurements the total cross-sectional areas of myelinated fibres (myelin sheath and axon) were calculated.

RESULTS

Remyelination in the regenerating ventral caudal nerve

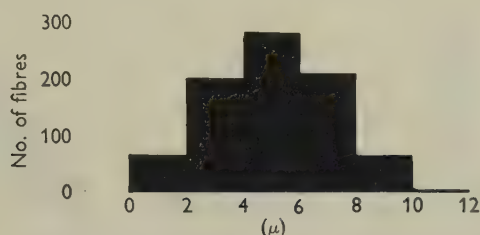
As reported recently (Gamble, 1958) the first indications of remyelination appear some 15 days after crush injury in the caudal nerves of rats kept at room temperature. Double, wavy osmophilic lines are suggestive of thin-walled myelin tubes of rather uneven calibre. These myelin tubes are usually associated with a Schwann cell nucleus which indents the wall, and successive segments are separated by gaps of rather variable extent. Such structures as these were seen in the six control animals killed from 15 to 19 days after crushing the ventral caudal nerve. In all three control animals killed after 21 days, however, recognizable Nodes of Ranvier were distributed regularly and at short intervals along the length of the majority of the fibres studied. The transition at the crush level was striking; the normal fibres, of various calibres and with myelin sheaths showing various internodal lengths, gave way to fibres of uniform calibre, with myelin sheaths of uniformly small size and short internodal length (Pl. 1, fig. 1). Nodes of Ranvier at regular intervals were not seen in the control specimens after survival periods of less than 21 days; they were, however, present on the majority of the fibres examined from the two pyronin-fed animals, and these animals had been killed only 14 days after crushing the nerve. It seemed that pyronin-feeding accelerated the onset of re-myelination after nerve crushing by approximately 1 week, although teased osmium tetroxide preparations are not a very suitable basis for quantitative studies. The sural nerve was used for more accurate estimates of the effect of pyronin.

Remyelination in the regenerating sural nerve

The sural nerve in the rat (Pl. 1, fig. 2) is remarkably constant in the number and size of the myelinated fibres that it contains. Counts and measurements in four normal specimens showed mean numbers and standard deviations of 815 ± 18 and 825 ± 8 , respectively, at the crush level and at the level subsequently examined. The total cross-sectional areas at these levels were 20.0 ± 1.6 and 19.4 ± 1.8 ($1000\mu^2$), respectively. A histogram showing the diameter size-frequency distribution of the myelinated fibres forms Text-fig. 1. It also shows the unimodal distribution characteristic of a sensory nerve, with the mode between 4 and 6μ .

The counts of myelinated fibres, irrespective of their calibre, from nineteen crushed and regenerating sural nerves are summarized in Table 1, and histograms showing the diameter size-frequency distribution of these myelinated fibres after the different survival periods form Text-fig. 2.

After 30 days survival the numbers of fibres present in the treated specimens differ from those in the controls but with only a low level of statistical significance ($P < 0.1$ and > 0.05). After 50 days survival the difference in numbers is more highly significant ($P < 0.05$), but by 70 days the numbers present in both groups are close to those found in normal nerves and no significant difference is demonstrable.



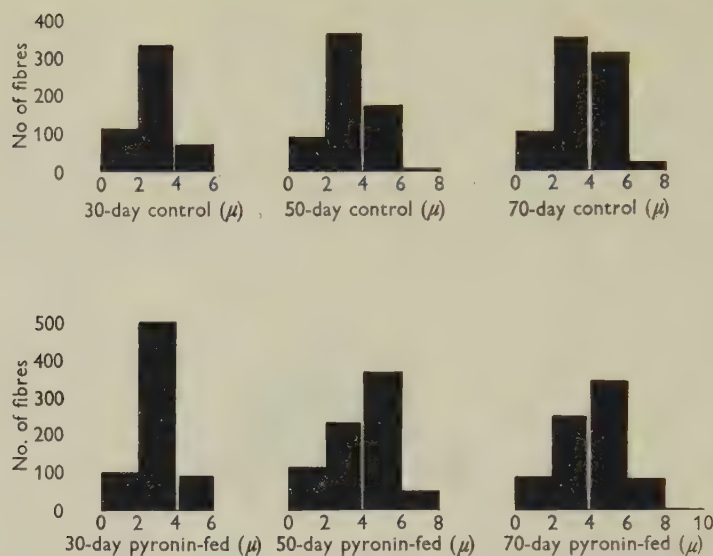
Text-fig. 1. Histogram representing diameter size-frequency distribution of myelinated fibres present in the normal sural nerve.

Table 1. The mean number of myelinated fibres (\pm S.D.) in the regenerating sural nerve after different intervals with and without 0.1 % pyronin to drink. The figures in brackets give the number of specimens in each case

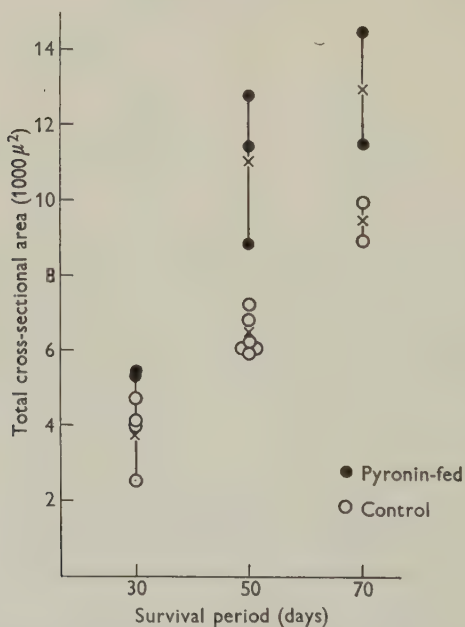
	Days of regeneration		
	30 days	50 days	70 days
Control	520 \pm 100 (4)	628 \pm 69 (6)	782 \pm 92 (2)
Pyronin-fed	690 \pm 30 (2)	766 \pm 99 (3)	821 \pm 14 (2)

The histograms in Text-fig. 2 show that after all three intervals of time, but more markedly after 50 and 70 days, there is a 'shift to the right' in the treated specimens. The effect of this 'shift to the right', combined with the greater number of fibres present in the 30- and 50-day treated specimens, is reflected in the total cross-sectional areas of myelinated fibres present in the various specimens as is shown graphically in Text-fig. 3. An analysis of variance (kindly made by Dr D. A. Sholl of University College London) of the cross-sectional areas measured after all three survival periods, grouped together, shows that untreated specimens differ from pyronin-fed specimens to a highly significant extent. Taking the 50-day specimens alone, the cross-sectional areas of the untreated specimens differ significantly from those of the pyronin-fed specimens ($P < 0.01$), but similar tests applied to the 30-day or to the 70-day specimens do not show statistically significant differences. However, in these latter groups the samples are very small.

It may be concluded that the remyelination of crush injured nerve fibres is accelerated in its onset, in the recovery of the normal complement of fibres and in at least the earlier stages of maturation by the administration of 0.1 % pyronin G. The possibility that pyronin acts by raising body temperature may be excluded;



Text-fig. 2. Histograms representing diameter size-frequency distributions of myelinated fibres present at various intervals in control and pyronin-fed animals after crush injury to the sural nerve.



Text-fig. 3. Graph to show total cross-sectional area of myelinated fibres (axon plus myelin) in sural nerve of rat after various survival periods in pyronin-fed and control animals. \circ or \bullet represents one specimen, \times represents the mean value for a group of similarly treated specimens.

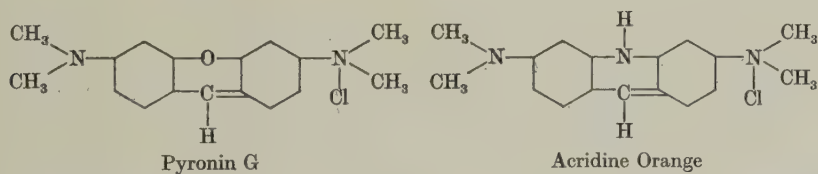
both intraperitoneal and peripheral subcutaneous temperatures have been measured by means of a thermocouple inserted into a hypodermic needle and the temperatures recorded lie within the range found in untreated animals.

DISCUSSION

The results and conclusions reported here are not inconsistent with Hoffman's (1952) hypothesis concerning the mode of action of pyronin. The attachment of pyronin to Nissl bodies with action upon their metabolic processes might be expected to result in accelerated axonal regeneration as well as in the accelerated axonal budding that he reported. There is, of course, no direct evidence that such a mechanism is involved in either process, nor even that Nissl bodies are the site of action of pyronin.

It has been shown (Duncan, 1934) that myelination will not ordinarily begin until the regenerated axon enlarges to a diameter of approximately $1-2\mu$. More axons are remyelinated in the pyronin-fed animals by 30 and by 50 days than in the control specimens. This may reflect a response in neuronal metabolism (possibly in the Nissl bodies) to pyronin such that the regenerating axons more rapidly achieve a diameter approximating $1-2\mu$. Inspection of our material (Pl. 1, figs. 3, 4) leaves little doubt that thicker myelin sheaths are present upon many axons in pyronin-fed specimens than upon axons of similar diameter in control specimens. Pyronin, then, seems to influence myelin formation at all the stages studied, but whether by direct action upon Schwann cells or indirectly through the mediation of the neurone it is not possible to say.

While the site of action of pyronin in accelerating nerve-fibre maturation remains so obscure its mode of action can hardly be less so, but a recently reported observation may be relevant. Beers, Hendley & Steiner (1958) have shown that polynucleotide phosphorylase activity may be inhibited or increased through the formation of complexes with Acridine Orange and polynucleotides; the same complexes are formed with ribonucleic acid and one of the complexes is formed with desoxyribonucleic acid. Acridine Orange and pyronin G are, respectively, acridine and



xanthene derivatives but as their formulae show (Conn, 1946) are otherwise remarkably alike. Moreover, preliminary fluorescence tests on Carnoy-fixed tissues confirm that both pyronin B and pyronin G have a special affinity for cell components which are known to contain nucleic acids. Sections of pancreas, for example, which have been immersed in a 0.05% solution of pyronin at pH 6.6 fluoresce strongly when activated with blue-violet light on the fluorescence microscope; the nuclear chromatin showed a bright yellow colour, while the chromidial substance of the cytoplasm presented orange-red fluorescence of somewhat lower intensity. The whole appearance was quite similar to that produced by Acridine Orange which,

under conditions of controlled pH, has been found useful for cytochemical differentiation of DNA and RNA (Armstrong, personal communication).

Possibly pyronin, like Acridine Orange, is capable of altering enzyme activity by the formation of complexes with polynucleotides and nucleic acids. There is as yet, however, no direct evidence of its site of action in accelerating nerve fibre regeneration. The study of injured nerves, both peripherally and in their cell bodies, with the fluorescence microscope may provide clues to the site of action.

SUMMARY

Pyronin has been fed as a 0.1% solution to young adult albino rats for periods ranging from 14 to 70 days after the ventral caudal nerve or the sural nerve had been crushed. Maturation, that is axonal growth plus remyelination, was found to be more advanced in these than in control animals' nerves.

We are indebted to Prof. F. Goldby for his advice and criticism throughout this work, to Dr D. A. Sholl for his help with statistical problems, and to Dr J. A. Armstrong for his investigation of the fluorescent properties of pyronin. We also wish to thank Mr C. A. Voyle for the photographs and Miss M. Rozier for many of our sections.

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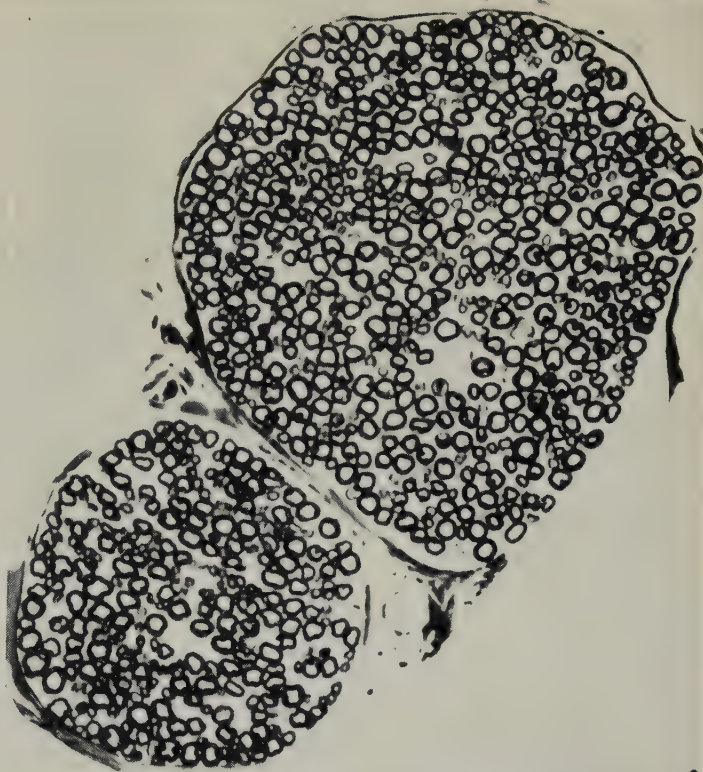
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EXPLANATION OF PLATE

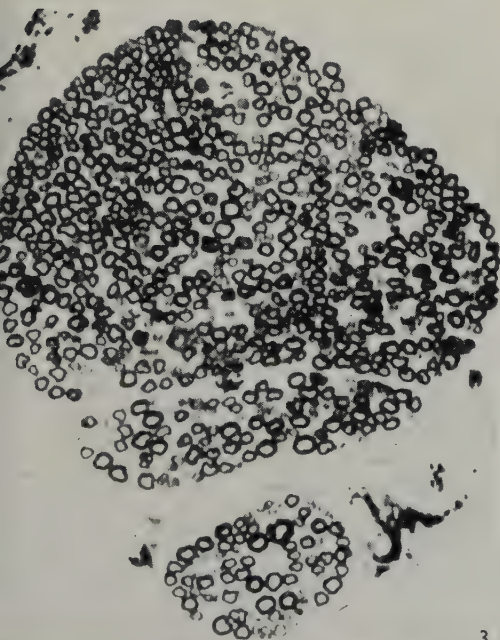
- Fig. 1. Teased preparation of ventral caudal nerve 21 days after crush injury (control specimen). The transition from the uninjured to the regenerated part is shown ($\times 150$).
- Fig. 2. Transverse section of normal sural nerve at the level studied in the experimental specimens ($\times 330$).
- Fig. 3. Transverse section of sural nerve 70 days after crush injury. Pyronin-fed specimen ($\times 330$).
- Fig. 4. Transverse section of sural nerve 70 days after crush injury. Control specimen ($\times 330$).



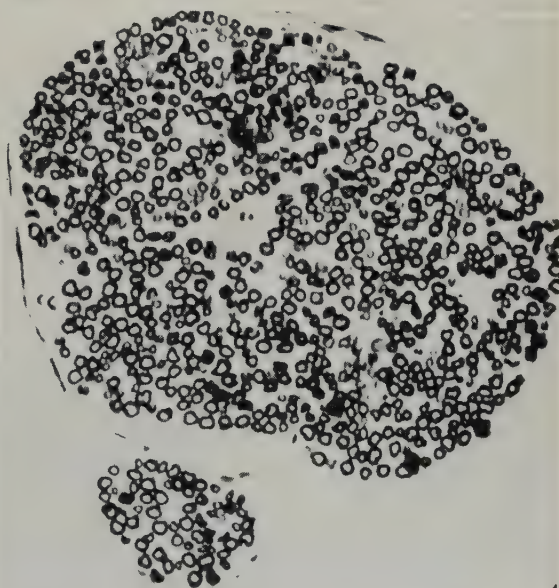
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THE POST-NATAL DEVELOPMENT OF THE RAT SUBMAXILLARY GLAND

By F. JACOBY AND C. R. LEESON*

Department of Anatomy, University College, Cardiff

The rat submaxillary gland has received considerable attention in recent years as an exocrine gland profoundly influenced by hormones, especially that of the thyroid. From the study of the relevant literature three surprising facts emerge: (1) the complex structure of the fully differentiated gland of the adult rat is often not fully appreciated nor, indeed, understood; (2) a confusing nomenclature exists concerning the different parts or segments which compose the glandular unit, as it repeats itself within a lobule; (3) the post-natal development of this gland does not seem to have been properly studied or described. Yet a glance at Figs. 1 and 2 of Pl. 1 is sufficient to make one realize what strides in development and differentiation the gland must obviously have made during the period which—so to speak—separates these two pictures. It is also obvious that an accurate and precise knowledge of the state of development of the gland is of particular importance in endocrinological studies, for which often very young animals are used. Moreover, certain histomorphological problems connected with the adult gland, but as yet unsolved, could well be clarified by a study of the post-natal developmental stages.

The present paper, therefore, deals in the main with the post-natal development of the rat submaxillary gland. But in order to make this account intelligible it is essential that the two other points mentioned above are considered first. Also any reference to the literature will be facilitated by an exposition given first of the structure of the gland as seen in the adult animal.

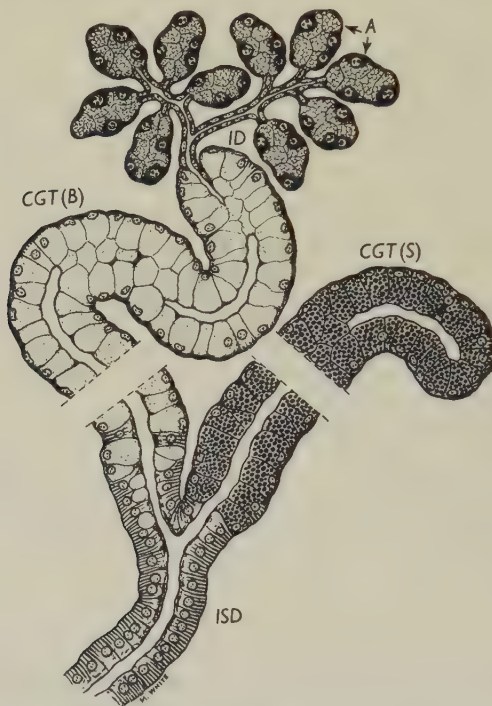
HISTOLOGY OF THE SUBMAXILLARY GLAND OF THE ADULT RAT

A lobule of such a gland appears as a compact structure containing various epithelial elements. Sorted out, there are four main parts which make up what one could call a glandular unit proceeding from end-pieces to an interlobular duct: (1) acini; (2) intercalated ducts; (3) convoluted tubules; and (4) intralobular 'striated' ducts (Text-fig. 1).

The acini are composed of pyramidal cells with a foamy basophil cytoplasm, the basophilia being strongest near the base of the cell, where it cushions the nucleus. There are no distinct secretion granules anywhere in the cytoplasm. The cells do not stain appreciably with mucicarmine or alcian blue. They give, especially after Susa or Orth fixation, a positive PAS reaction, which is, however, much less strong than that of the mucous cells of the neighbouring major sublingual gland. Fine intercellular canals (Sekretkapillaren) can be detected. The units are so compact that they deserve the name 'acini', and should not be called alveoli, although they

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are not always spheres but frequently elongated ovoids. Myo-epithelial cells (basket cells) are present embracing the acinar cells; they are not conspicuous in ordinary preparations, but can be clearly demonstrated by means of the alkaline phosphatase reaction (Pl. 1, fig. 3) (Leeson, 1956; Leeson & Jacoby, 1957). Several such acini are connected to a branching intercalated duct, a narrow tube lined by flat epithelial cells. Here, too, occasional basket cells occur, though they are less complex in their ramifications than those of the acini. These intercalated ducts are not very easily seen in the adult gland, as they are lying compressed between adjacent bulging structures, around which they often curve (Pl. 1, fig. 2; Pl. 4, fig. 25). A single intercalated duct becomes continuous with the next element, the convoluted



Text-fig. 1. Diagrammatic representation of the different epithelial portions of a glandular unit of the adult rat submaxillary gland. *A* = acini, *ID* = intercalated duct, *CGT(B)* = convoluted granular tubule after alcoholic Bouin fixation, *CGT(S)* = convoluted granular tubule after Susa fixation, *ISD* = intralobular striated duct. Myo-epithelial cells are not shown. The *CGT(S)* is also continuous with an intercalated duct; but this has been omitted from the diagram.

tubule. The transition from the low epithelium to the high columnar type of the convoluted tubule is fairly abrupt, but the point of entrance (junction) is rarely seen clearly. In fact, Loewenthal (1908), who gave the first detailed microscopic description of this gland, was unable to convince himself of this junction, and also Tupa (1926) in his otherwise exquisite account experienced difficulties about this point and mentions a lateral as well as an end-to-end junction. In our material, we have seen only the latter and cannot confirm the existence of the former. There are, however, variations of this junction which will be discussed later on. In this paper,

we shall, not only for convenience but also for reasons which will become clear later, refer to this junction throughout as the intercalated duct-striated duct junction, although in the adult it is, strictly speaking, an intercalated duct-convoluted tubule junction.

The convoluted tubule, which, in the adult gland, is perhaps the most striking epithelial component, is part of a widely branching tubular system. It is characterized by tall columnar epithelial cells with their nuclei lying near the base of the cell; below the nuclei short acidophil basal 'striations' can sometimes be discerned. The appearance of the cells above the nucleus depends on type of fixation and staining (see Text-fig. 1). For instance, after alcoholic Bouin fixation and staining with haematoxylin and eosin they will appear rather empty, almost vacuolated (e.g. Pl. 1, fig. 2; Pl. 3, fig. 16); and only high magnification will reveal the presence of dispersed fine, almost unstained granules (Pl. 3, fig. 17). On the other hand, after fixation with Susa, Zenker or Orth, they are more or less packed full with large distinct granules of unequal size, which stain intensely with, amongst others, Altmann's acid fuchsin and orange G (Pl. 3, fig. 18; Pl. 4, fig. 19). They give only a very weak PAS reaction and do not stain with either mucicarmin or alcian blue. These granules have been interpreted as secretion granules, and they can be found also within the lumen, presumably after discharge from the cells. Hence, these convoluted tubules are also referred to as 'serous' tubules or 'granular' tubules; and it is these segments which are so profoundly affected by hormones.

Such a branching convoluted 'granular' tubule eventually becomes continuous with the fourth segment, the 'striated' intralobular duct, well known in its appearance. It has high columnar cells with more or less centrally or even apically placed nuclei and a pronounced basal striation ('Streifenstücke' of the German authors). The junction between the two segments is sometimes abrupt, and the overall diameter of the tubule suddenly narrows down; or else the transition is more gradual, and over a certain stretch cells typical of either segment are intermingled (see Text-fig. 1 and Pl. 3, fig. 18).

These are the main four epithelial elements forming the bulk of a lobule. Adding to the complexity of the gland, there are found, not infrequently, a few scattered, probably aberrant, typical mucous acini. These are more often present in lobules adjacent to the neighbouring major sublingual gland than elsewhere and are identical with the acini of the latter. They were already noticed by Loewenthal (1908).

Finally, in some of the glands, complexes of narrow acinar and/or tubular formations, quite unlike anything else in the lobule, were observed. They form, as it were, little islands, yet connected to the general duct system, and their nature is quite obscure. These last two epithelial components are mentioned for the sake of completeness only and will not be referred to further.

Coming now to point (2), viz. the confusing nomenclature existing in the literature pertaining to the different segments of the gland—not only in the rat, but also in other species whose submaxillary gland has a corresponding composition, e.g. mouse, shrew, golden hamster—one has to consider the confusion in terminology both as to form and as to presumed function.

Thus, the acini are referred to as glandular tubules by Stormont (1932) and Duthie (1934), as lobes by Kurtz (1954), as tubulo-alveolar end-pieces by

Boerner-Patzelt (1955-56); and the convoluted granular tubules are referred to as acini by Honda (1927), as terminal tubules by Fekete (1941) and as serous alveoli by Pease (1956). This slackness in nomenclature is not only confusing, but often indicates a complete misinterpretation of the architecture of the gland. Probably thanks to Tupa's paper (1926), confusion as to form hardly exists in the French literature.

Regarding the functional interpretation of the same two main elements, the majority of workers assigns to the acini the term 'serous'. Grad & Leblond (1949) and Leblond (1950), however, declare them as 'mucous' and 'atypical mucous', respectively, in view of the fact that, after formol-bichromate fixation, they give a positive PAS reaction and stain metachromatically with toluidine blue. Gautier & Diomedea-Fresa (1953) also call these cells 'mucous'. Schaffer (1908) had named them 'sero-mucinous', and Boerner-Patzelt (1955-56) concludes that they are more mucous than serous. Stormont (1932) introduced the term 'special serous' (see below). Other workers do not commit themselves (Tupa, 1926; Hillarp, 1949).

A decision as to the nature of the acinar cells is, indeed, difficult to make, but there is something to be said for considering them to be 'mucoid' rather than serous; there is, however, hardly any evidence for calling the heavily granulated cells of the convoluted tubules 'mucous'. Yet they are referred to as such by Kurtz (1954) and by Rutenburg *et al.* (1958) and, with some reservation, by Burkl (1953) and as 'mucoid' by Burstone (1956) and by Glenner & Lillie (1957).

Stormont (1932) in an attempt to classify the non-mucous cells of salivary and other glands introduced the term 'special serous' as distinct from sero-zymogenic. The latter term, characterizing cells with distinct zymogenic granules, basal chromidial substance and intercellular secretory capillaries, is, according to Stormont, applicable only to a very few distinguished members of this class of cells, e.g. the acinar cells of the pancreas, peptic cells of the stomach and some selected crescent cells of certain salivary glands. The unfortunate result is that such heterogeneous types of cell as the acinar cells of the rat submaxillary gland and the 'granulated' cells of its convoluted tubules are both referred to as 'special serous'.

In view of this confusing and unsatisfactory state of affairs we shall, for the purpose of the present paper, follow Tupa's example and call the two elements *acinar cells* and *cells of the granular tubules*, respectively.

Not only has the functional nature of the granular tubules been subject to speculation, but even their precise position within the gland, their anatomical and developmental nature, has not been clearly recognized or established. Loewenthal (1908), who was probably the first to pose the question whether these tubules were a special gland somehow connected with the duct system or, in fact, transformed intralobular segments of this system, was unable—from his study of the adult gland—to answer it. Tupa (1926) did not discuss the origin of the tubules but merely described them as part of the duct system. Gabe (1950*a*) refers to the concept of the tubular granulated segments representing a specially differentiated portion of the excretory canal as a hypothesis. Shafer & Muhler (1956) say vaguely that the granular tubules *appear* to lie between the acini and the intralobular ducts. Vaguer still, inaccurate and incomplete is the account and diagram given by Bixler *et al.* (1958). Only Screebny *et al.* (1955) state that 'the cells of the intralobular ducts... undergo a slow transformation into secretory cells'.

Another point, which may be clarified by the study of the post-natal development of the gland, is the nature of the intercalated duct-striated duct junction, which up to date has puzzled most investigators and has remained somewhat obscure.

THE POST-NATAL DEVELOPMENT OF THE GLAND

During the study of the extensive literature relating to this gland we could find only one paper concerned with the post-natal development of the gland in rats. Screebny *et al.* (1955), though primarily interested in the proteolytic activity of extracts of the submaxillary gland of young male and female rats, give also a brief morphological account of the gland at five stages between 15 and 150 days, but this account is very general, superficial and in parts inaccurate. They begin with the curious statement that 'the general morphological pattern remains the same in all stages'. Later they do, however, refer rightly to the acinar formation running ahead of that of the convoluted tubules up to day 60. Further comments on this paper are best postponed until our own findings have been given.

MATERIAL AND METHODS

Seventy-six male and female rats aged from 1 day to 6 months were used. Up to 12 weeks the series includes glands differing in age by a week, thereafter by a month. For each stage at least three glands were examined, but for the more important stages, between 1 week and 8 weeks, up to ten glands were used. The animals were killed by a blow on the head, and the submaxillary gland or glands removed together with the major sublingual gland (retrolingual of Ranvier, 1886), which incidentally served as a convenient control for mucin stains.

The following fixatives were employed: 80 % alcohol, alcoholic Bouin, Susa and Orth. Serial sections from the paraffin-embedded material were cut at 6μ . Some sections from individual glands were mounted separately; in most instances, a series of sections from glands of different ages were mounted in chronological order on the same slide. This was done not only to facilitate histological comparison of the various stages, but also to insure similar staining conditions throughout a series in order to put the assessment of differences in staining properties on a firm basis. The following staining techniques were used: haematoxylin and chromotrope, haematoxylin and eosin, azan stain, Altmann's acid fuchsin, Mayer's mucicarmine, alcian blue, the PAS reaction, Best's carmine (before and after saliva digestion) and metachromatic stains, such as toluidine blue, methylene blue and azure A.

RESULTS

We shall use the terms 'proximal' and 'distal' with reference to the flow of the secretion; hence the opening of the major excretory duct in the mouth cavity is the most distal point. But for the designation of the glandular end-pieces as formed during foetal life we shall use the expression 'terminal tubules'. These are not found as such in the adult gland, but are—as will be seen—transitory structures.

0–2 days post-natal (Pl. 1, figs. 1, 4, 5). Compared with the adult gland the organ is still in a relatively rudimentary state. Lobulation is just recognizable, but a fair amount of loose mucoid connective tissue still pervades the lobule. The larger inter-

lobular ducts with wide lumina are often seen as isolated units surrounded by more connective tissue. They have a columnar epithelium with a definite basal striation. The intralobular ducts have narrower lumina, a low columnar or cubical epithelium, the cells of which are so small that their nuclei appear crowded together; only in some cells is a low basal striation discernible. When cut longitudinally, these ducts can be seen to be continuous proximally with somewhat narrower ducts (Pl. 1, fig. 5), whose epithelium is lower still and whose equally 'crowded' nuclei are often elongated parallel to the direction of the duct. These narrow ducts, in turn are continuous with branching terminal tubules. The columnar or narrow-pyramidal cells of these terminal tubules are characterized by strongly eosinophil, PAS-positive (Pl. 1, fig. 4), rather fine granulations and by round basally situated nuclei. The extent of the granulation is so great that, in haematoxylin-chromotrope or haematoxylin-eosin preparations, a basophilia cannot be discerned, but in sections stained with neutral red or toluidine blue there appears to be some chromidial substance around the nucleus. The lumen of these terminal tubules can often be seen clearly. At some places at the periphery of the tubules one or two paler epithelial cells are 'budded' out (Pl. 1, fig. 5); these, too, are fundamentally eosinophil and often also show granulation. In an azan-stained section (after Susa fixation) the granules of the terminal tubules and also of some of the cell-buds stain in varying shades of blue, but within any cell the intensity of the stain is uniform, and the discreteness of the granules is brought out particularly well. Many mitoses are present in the terminal tubules (Pl. 1, fig. 1), including buds, and in all segments of the duct system.

Interpretation. The place of narrowing of the intralobular duct is, in our opinion, the site of the future intercalated duct-striated duct junction, and hence these two segments are, at this stage, already determined and mitotic proliferation takes care of their future growth. The nature of the 'budded' cells is more difficult to interpret. They have obviously arisen either by a process of differentiating mitosis or simply by movement from the cells of the terminal tubule, with which they share some features, such as eosinophilia and granulation, though the latter is often reduced. We believe that they are the immediate forerunners of the definitive acinar cells.

1 week (Pl. 1, fig. 6; Pl. 2, fig. 7). The gland appears somewhat more compact, but there is still plenty of loose mucoid connective tissue separating lobules and also the glandular units within the lobule. Basal striations in the cells of the inter- and intralobular ducts are more distinct and more widespread. Intercalated ducts are more clearly suggested by narrowing of the most proximal part of the intralobular duct system and by the arrangement of their nuclei. The fine granules of the terminal tubules are again found to be eosinophil and, in azan preparations, aniline blue-positive, but variation in staining intensity from cell to cell is more marked. The same holds for the PAS reaction (Pl. 2, fig. 7). Individual cells budded out from the terminal tubules are again seen. They are pale and may or may not contain granules with staining reactions similar to those of the terminal tubules. Numerous mitoses are present in the terminal tubules; occasional mitoses are seen in all segments of the duct system.

2 weeks (Pl. 2, fig. 8). Increased compactness of the lobules is now very definite. There is only a moderate intensity of staining of the granules of the terminal tubules both with eosin or chromotrope and with aniline blue. Around the terminal

tubules cellular buds are now seen frequently; they appear either as individual cells or even as small crescents; though pale, they have now a distinct general basophilia and no longer contain granules. In some specimens the formation of crescents is so advanced as to make the terminal tubules appear as 'centres', and there is no doubt that the crescents represent the future definitive acini. Definite intercalated ducts are present and are clearly continuous with the striated intralobular ducts, which, at places, show convolutions. Mitoses are fairly frequent in buds and terminal tubules, but are seen only occasionally in ducts.

3 and 4 weeks (Pl. 2, figs. 9, 10). Acini and terminal tubules with crescents now dominate the picture outnumbering by far the striated ducts; acini, crescents and buds, in turn, outnumber the remnants of the terminal tubules or 'centres'. The acinar cells have a somewhat foamy appearance with a well-marked basophilia. The eosinophilia of the granules of the terminal tubules and 'centres' is increased, especially so at 4 weeks, but the staining of the granules with aniline blue (after Susa fixation) is very intense at both periods, which makes these structures stand out most strikingly. Secretory material with similar staining reactions is also seen in the lumina of terminal tubules and intercalated ducts. In some places, the cells comprising the centres are reduced in height and their nuclei flattened at the bases; and here and there where these centres link up with intercalated ducts, 'centro-tubular' nuclei are seen (Pl. 2, fig. 11*b*). Convolutions of the striated ducts, noted already at 2 weeks, are now more advanced. Mitoses are most frequent in acini, buds and crescents, but are also seen in terminal tubules and intercalated ducts, more rarely in other ducts.

5 and 6 weeks (Pl. 2, figs. 11*a*, 12; Pl. 3, figs. 13, 14). Acini predominate by far over ducts. Centres are much reduced, both in number and size; in some specimens they are no longer present. In others they can be seen to be flattened, still containing granules staining deeply blue with aniline blue, and now forming the most proximal branching system (2nd order) of intercalated ducts. Each little branch is linked to an acinus which developed from an individual crescent. Of particular interest is the junction between an intercalated duct (1st order) and the proximal end of the now convoluted striated duct. It should be emphasized that, in contrast to the state of affairs in the adult gland, this junction is easy to see in these earlier developmental stages. The intercalated duct enters, as it were, the pole, sometimes with a few of its flat epithelial cells (nuclei) lying right in the interior of the striated duct, forming a kind of 'intussusception' (Pl. 4, figs. 22-25). Very often the most distal part of the intercalated duct curves round the first bend of the convoluted striated duct. The striated ducts show, in some of the specimens, the earliest signs of change into 'granular'-tubules; i.e. beginning apical vacuolization (after alcoholic Bouin fixation) of scattered cells (Pl. 2, fig. 12), or accumulation of coarse yellow or orange granules after Susa fixation and azan staining. This change is confined to the more proximal segments of the intralobular striated duct system. The acini still show considerable mitotic activity; mitoses are less frequently seen in 'centres' and all ducts.

7, 8, 9, 10 weeks (Pl. 3, figs. 15, 16). The convolutions of the branching system of the intralobular striated ducts is now well displayed, although the space they occupy is still less than that occupied by acini. Centres with aniline blue-staining granules are

not present any more, i.e. they all have by now been transformed into the 2nd order intercalated ducts. The intercalated duct-striated duct junctions show frequently the 'intussusception' described above. From the seventh week on vacuolization or granulation, respectively, is definitely under way in the proximal portions of the convoluted ducts and, by the ninth week, has become fairly extensive. The distribution is at first erratic, and changed cells alternate with others as yet unchanged. Later whole continuous rows of changed cells are encountered. Some cells appear pale and 'empty' apically, others have already produced definite granules which stain yellowish-orange with the azan stain, and which vary in size from fine ones to very coarse ones (up to 3μ or more) (Pl. 4, fig. 19 shows this in a mature tubule); many cells are stuffed full with these granules, and then their height and width are increased. In addition (in azan preparations) many of these cells have scattered, red-staining granules, also of varying sizes. Their occurrence is constant, but the significance of the difference in staining reaction is not understood. With the accumulation of granules in these cells their nuclei tend to lie in the basal third of the cell, sometimes flattened; and the basal striation is very much reduced or no longer discernible. This change, though predominantly localized in the proximal portions of the convoluted ducts, seldom involves, at these stages, their very 'tips' (intercalated duct-striated duct junction). Mitoses are rare, but when seen, are more frequent in ducts than in acini (Pl. 4, fig. 20).

3-6 months (Pl. 1, fig. 2; Pl. 3, figs. 17, 18; Pl. 4, fig. 19). The appearance of the gland is now very similar to that in the adult animal, already described. The large, highly convoluted, heavily granulated tubules dominate the picture. The granulation commonly extends right down to where the duct leaves the lobule, and often the change from the wide granulated portion to the somewhat narrower striated portion is fairly abrupt. Now, even some cells of the 'tips' are more or less filled with granules (Pl. 4, fig. 25); and an 'intussusceptional' junction is rarely found, as if with enlargement of the tubular cells the invaginated flat cells of the intercalated duct had been pushed out. This enlargement of the cells at the 'tip' of the convoluted tubules is often unequal or one-sided with the result that the point of junction appears to lie somewhat eccentrically to the very pole (Pl. 4, fig. 25). Such an arrangement could have caused Tupa (1926) to talk of a lateral connexion. But it should have become clear from the description of the various developmental stages that fundamentally the junction is an end-to-end one. Various factors combine to make its analysis difficult: (a) the convolution of the granular tubule, (b) the branching, in a Y- or T-shaped fashion, of the intercalated duct which frequently occurs close to the junction, and (c) the curved deviation of the intercalated duct from a straight course (Pl. 4, figs. 21-25). Sometimes instead of an abrupt change from flat epithelial cells to high-columnar ones there is a gradual increase in height involving three or four cells.

At 4 months duct mitoses were still observed.

The more important staining reactions of the various structures of the gland, both during its post-natal development and in its adult form, are summarized in Table 1, in which for comparison, a column for the acini of the major sublingual gland is included.

The table shows clearly the distinct difference between the granules of the terminal tubules and those of the granular tubules. The granules of the terminal tubules give a positive PAS reaction; this reaction is even stronger in the definitive acini, which might indicate that the secretion, in granular form, of the terminal tubules is akin to the material formed later in the acini. This adds a cytochemical relationship to the developmental one between these two elements.

Table 1

	Submaxillary gland			Sublingual gland	
	Granules of terminal tubules and 'centres'	Acini*	Contents of intercalated ducts	Granules of granular tubules	Acini
Eosin or chromotrope	++	—	—	+	—
Aniline blue†	+++++	(+)	+	—	(±)
Orange G‡	—	—	—	+++	—
PAS‡	+	++	(+)	(±)	+++
Best carmine (saliva-resistant)	+	—	—	—	—
Mucicarmine	—	(+)	—	—	+++
Alcian blue	—	(+)	—	—	+++
Metachromasia	—	(±)	—	—	(+)

* These do not include the aberrant mucous acini, which give the same staining reactions as do those of the sublingual gland.

† As used in the composite azan stain.

‡ Especially after Susa or Orth fixation.

It should be noted that only mast cells—always present in these sections—were found to give an alcohol-resistant metachromasia, but the much weaker, though definite metachromasia given by the acini of both the submaxillary and the sublingual glands was quickly removed, more or less completely, by alcohol. Staining of the acini with mucicarmine or alcian blue was negligible. Thus they do not qualify for the term 'mucous' nor, in view of the absence of secretory granules, for the term 'sero-zymogenic', though they are rich in chromidial substance. Further cyto- and biochemical work will be necessary to decide the nature of the secretion both of the acini and of the granular tubules.

DISCUSSION

The most striking points brought out by this investigation are: (a) the absence of acini at birth; (b) the part played in the development by the terminal tubules and (c) the relatively late differentiation of the granular tubules. Another interesting point, already noted by Screebny *et al.* (1955), is the considerable length of time required for the gland to reach full maturity; this is usually not attained before the third or fourth month, that is well past the pubertal stage of the animal.

This post-natal developmental period clearly falls into two phases, the first one from birth to approximately 6 weeks and the second thereafter. During the first phase acinar formation, clearly under way at 2 weeks, is in the foreground. Definitive acinar cells arise in the form of buds from the terminal tubules. This, together with mitotic proliferation of the 'budded' cells, results in the formation first of multiple crescents and eventually of acini. In this process the terminal tubules become first the 'centres' which, as previously described, are a quite remarkable

feature of the gland from 3 to 5 weeks, and then, by reduction in size of cells and loss of granules, become intercalated ducts of a 2nd order which form the twig-like links between the emancipated acini and the original intercalated ducts of the 1st order. Mitotic activity is present during this first phase also in the whole of the duct system and accounts for lengthening of its units and convolution of parts of this system. Screebny *et al.* (1955) do not seem to have recognized what we have called 'terminal tubules' and later 'centres'. This may be partly due to the fact that the earliest stages they studied were 15 days old; though it is surprising that they failed to see the centres at the 30 days' stage when they are most impressive. They state that the acini arise from those intralobular ducts which later transform into the granular tubules, which is obviously not so. Intercalated ducts are entirely omitted from their account.

During the second phase acinar growth gradually subsides and growth and differentiation of the intralobular striated ducts become more and more marked.

Some quantitative studies were made to supplement the descriptive histological observations. We measured in thirty-four specimens (covering the period from day 1 to week 24) diameters of the intralobular striated ducts and, in twenty-one specimens, from 6 weeks on, in addition, diameters of the granular tubules and also of the acini, which only then have achieved distinct separate existence. The mean values are given in a condensed form in Table 2 with the ranges in brackets.

Table 2. *Diameters in μ*

	Intralobular striated ducts	Granular tubules	Acini
1 day	25.3 (20-30)	—	—
1-3 weeks	27.2 (23-35)	—	—
4-5 weeks	30.5 (25-38)	—	—
6-7 weeks	30.2 (25-35)	33.1 (28-40)	26.4
8-10 weeks	31.7 (25-40)	36.9 (30-45)	26.3
12-14 weeks	35.4 (28-40)	43.4 (35-55)	26.8
4-6 months	35.6 (28-40)	44.5 (38-50)	26.4

They show that the diameter of the intralobular striated ducts increases steadily from birth to the third month. As the size of the lumen increases only by 2-3 μ , this growth is mainly due to increase in height of the constituent cells. The granular tubules, only measurable from the time they become recognizable as such, that is 6 weeks at the earliest, have greater diameters which increase to a greater extent, also up to the third month. Accumulation of granules within the cytoplasm naturally contributes to the ever increasing height of the cells. The acini, once formed, have a fairly constant size.

We also estimated the relative space occupied within a lobule by tubules and acini, respectively, the so-called T/A ratio, where T covers all intralobular duct segments, except the intercalated ducts. For the purpose of this estimation we used a series of Susa-fixed specimens. Sections stained by means of a modification of Wilder's silver impregnation for reticular fibres were found particularly suitable, giving a striking contrast between ducts and acini. Lobular areas were photographed; from the enlarged prints ducts and acini were cut out, collected separately and weighed. Table 3 gives the T/A ratios for the different ages, thus studied, and also the proportion of the total space occupied by tubules. The results confirm

quantitatively the visual impressions obtained. From 6 weeks on the ducts and/or tubules gain on the acini, and in the fully developed gland they take up half, or even more, of the total lobular space. This is due not only to increase in size of cells and accumulation of granules, but to a considerable degree also to cell proliferation. There is from 6 weeks on a shift in mitotic activity from acinar to duct cells. These duct mitoses were seen both in unchanged cells and in cells already containing secretory granules.

Table 3

Weeks	T/A	T as proportion of total
6	0.27	0.21
7	0.33	0.25
9	0.49	0.33
13	0.53	0.35
16	1.10	0.52

Apart from their morphogenetic role the terminal tubules, and centres respectively, also seem to possess secretory activity, judged by their rich granulation and the fact that material with staining reactions similar to those of the granules is found in related lumina and ducts. This secretory activity is only transitory and has ceased by the seventh week. At that time acini are fully formed ready to take over, and also the secretory changes in the convoluted tubules have commenced. In this connexion it is worth recalling the work of Plagge (1938) who found that removal or duct ligation of all four salivary glands (the two submaxillary and the two major sublingual) results in death of newborn rats within 5 days; but if only one of these glands is left intact, the animal will survive. This survival may not only be connected with the secretory function of the terminal tubules, but also with that of the striated ducts. The latter, as is well known, are assumed to be concerned with water transport; and it should be noted that striations are already differentiated at birth.

The formation of the intercalated ducts, 1st and 2nd order, and the nature of the intercalated duct-striated duct junction have already received sufficient comment. As to the granular tubules, the study of the present series proves beyond doubt that it is the convoluted branching system of the intralobular ducts whose cells—from about the sixth week on—are gradually transformed into secretory granule-producing cells; that this transformation, though somewhat erratic at first, proceeds fundamentally in a proximal-distal direction, except for the very 'tip' (i.e. place of junction with intercalated duct), where the change sets in relatively late. The transformation can eventually become so complete that only the most distal short segments remain unchanged, just prior to where they join the interlobular ducts.

Milstein (1950) studied regeneration in the rat submaxillary gland. Two of his observations are of interest in relation to our study. He describes the newly formed acini as staining deeply with eosin and with Altmann's acid fuchsin, and on his photographs they appear heavily granulated. One wonders whether these were not regenerated terminal tubules rather than acini, as he goes on to say that later the acini were found to be basophil. Secondly, he notes that in a regenerated lobule

the granular tubules were not in evidence before the eighth week after operation, which points to a time lag similar to that found in this study of the normal post-natal development.

Whilst the sex dimorphism of the mouse submaxillary gland has, since the work of Lacassagne (1940*a*), been well established and recognized, there is less clear-cut, or even conflicting, evidence on this point concerning the rat. Hammet (1923) noted that the submaxillary gland of rats was, at 150 days, relatively heavier in males than in females, and that lack of thyroid completely inhibited growth of the gland in females, whilst only markedly retarding it in males. Lacassagne (1940*b*) observed that injection of testosterone into female rats caused increase in diameter of the granular tubules (up to 53μ), though untreated animals (male and female) did not show significant differences in tubule diameters. On the other hand, Grad & Leblond (1949) reported significantly larger tubule diameters in males than in females, at least at day 39 and 150. This was not confirmed by Arvy, Debray & Gabe (1950). Similarly, Raynaud & Rebeyrotte (1950) did not find a sex difference in rats of amylase activity in submaxillary gland pulp, in contrast to that found in mice (Raynaud & Rebeyrotte, 1949, 1950). Screebny *et al.* also deny the existence of a structural sex difference of the rat submaxillary gland, but observed a functional one: proteolytic activity of the glandular extract was markedly higher in males than in females. Our own material allows of the following contribution to this problem. There were no striking sex differences with regard to pace and extent of the maturation of the gland. In one series we measured the diameters of intra-lobular striated ducts and granular tubules on specimens from litter mates (male and female) which had had the same technical treatment. Up to and including 7 weeks there was no difference in duct or tubule diameters, but from 8 weeks on the diameters of both these structures were on the average $3-4\mu$ (range $1.5-7.4\mu$) larger in the males than in the females, and in no instance did the figures for the female exceed those for the male. These results are, at least in part, in agreement with those of Grad & Leblond (1949).

The analysis of the mechanism of this sex dimorphism revealed the prime importance of the thyroid gland (Grad & Leblond, 1949; Raynaud, 1950; Gabe, 1950*b*; Arvy & Gabe, 1950*a*). Injections of thyroxine or feeding desiccated thyroid stimulate growth and granulation of the convoluted tubules (Arvy & Gabe, 1950*a*; Shafer & Muhler, 1956); thyroidectomy and thiourea have the reverse effects (Arvy & Gabe, 1950*a*; Arvy *et al.* 1950). Oestrogenic hormones have an action antagonistic to thyroxine (Arvy & Gabe, 1950*b*), whereas testosterone acts synergistically (Grad & Leblond, 1949; Shafer & Muhler, 1956). In addition, the existence and intervention of an hypophyseal factor has been evoked. However, in the work dealing with these aspects of the subject, the long drawn-out development of the gland has not always been properly taken into account. Grad & Leblond (1949) refer to, and even give measurements of, the granular tubules at a time (10 days and 39 days) when they are not yet differentiated. Gabe (1950*b*), Eartly & Leblond (1954) and Clark, Shafer & Muhler (1957) studied the effect of thyroxine after hypophysectomy in some series of animals, whose ages at the end of the experimental periods were such that granular tubules could hardly have been expected to have developed, or only very little so. They concluded that hypophysectomy causes

atrophy of the granular tubules, when it would be more exact to say that it causes failure of tubular differentiation or development, at any rate at some of the stages studied.

SUMMARY

1. The post-natal development (from day 1 to week 24) of the rat submaxillary gland has been studied.

2. A brief account is given first of the architecture of the adult gland and attention drawn to the confusing terminology in general use as regards its epithelial components.

3. At birth, acini are not yet formed. The intralobular ducts, already segmentally determined and to some extent differentiated, end in a branching system of terminal tubules, whose cells are highly granulated.

4. From the first week on, acinar cells are 'budded' off from the terminal tubules and proliferate mitotically, gradually forming crescents around the terminal tubules, which become 'centres'.

5. These 'centres', by reduction in size of their cells and loss of granules, are, from the fifth week on, transformed into intercalated ducts (2nd order), and all crescents become acini.

6. At 6 weeks, the phase of acinar development combined with the regression of the terminal tubules is practically complete, and growth and differentiation of the intralobular striated ducts come into the fore, coupled with a shift in mitotic activity from acini to duct cells.

7. With the elaboration of secretion granules these ducts become transformed (in a proximal-distal direction) into the convoluted granular tubules. This transformation is often still incomplete at 4 months.

8. The hitherto obscure intercalated duct-striated duct junction is analysed and described in some detail.

9. Data are given for duct and tubule diameters and tubular/acinar ratios during the post-natal development of the gland.

10. There was no sex dimorphism of the developing gland up to 7 weeks; thereafter duct and tubule diameters were greater in the male than in the female.

11. The importance, particularly for endocrinological work, of the knowledge of the long drawn-out development of the gland is stressed.

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ADDENDUM

A correction which we had proposed for our abstract (Light- and electron-microscopic observations on post-natal stages of the rat submaxillary gland. By C. R. Leeson and F. Jacoby; this Journal (1958), **92**, 659) was unfortunately incompletely reproduced. For 'special serous' read each time 'granular' and for 'secretory ducts' read 'striated ducts'.

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EXPLANATION OF PLATES

All the figures are of rat submaxillary glands, the majority of which was fixed in alcoholic Bouin. This was found to give a convenient degree of shrinkage separating the glandular units thus helping in their analysis and display.

PLATE 1

- Fig. 1. 1 day; fix. alc. Bouin, haematoxylin (Hx.) and chromotrope (Chr.) The lobule consists mainly of branching terminal tubules with granulated cells, six of which are in mitosis. Two intercalated ducts are also present, one in L.S., one in T.S. (↓). × 290.
- Fig. 2. Adult (6 months); fix. alc. Bouin, Hx. and Chr. Note contrast to Fig. 1. The pale curved elements are the convoluted granular tubules. There are two intralobular striated ducts near the right edge in the middle and one interlobular duct at the very top. Two intercalated ducts are arrowed. Remaining units are acini. × 180.
- Fig. 3. Adult; fix. acetone, Gomori's alkaline phosphatase reaction, incub. time 1 hr., to show branching myo-epithelial (basket) cells. × 890.
- Fig. 4. 1 day; fix. alc. Bouin, PAS reaction and Hx., to show the fine granules in the cells of the terminal tubules, which are PAS-positive, and a branching intralobular duct. × 335.
- Fig. 5. Newborn; fix. alc. Bouin, Hx. and eosin. Intralobular duct narrowing down to an intercalated duct; also terminal tubules and cell 'buds' (↓). × 350.
- Fig. 6. 1 week; fix. alc. Bouin, Hx. and eosin. A system similar to that of Fig. 5. Cell buds arrowed. × 560.

PLATE 2

- Fig. 7. 1 week; fix. Susa, PAS reaction and Hx. The reaction is of varying intensity in the cells of the terminal tubules and cell buds. Note mitoses in intercalated duct and terminal tubules. × 535.
- Fig. 8. 2 weeks; fix. alc. Bouin, Hx. and Chr. One intralobular striated duct in T.S., one intercalated duct in L.S. joined to a terminal tubule showing mitosis. Note weakly acidophil granulation of the terminal tubules. Many acinar cell buds and crescents, in places isolated. × 560.

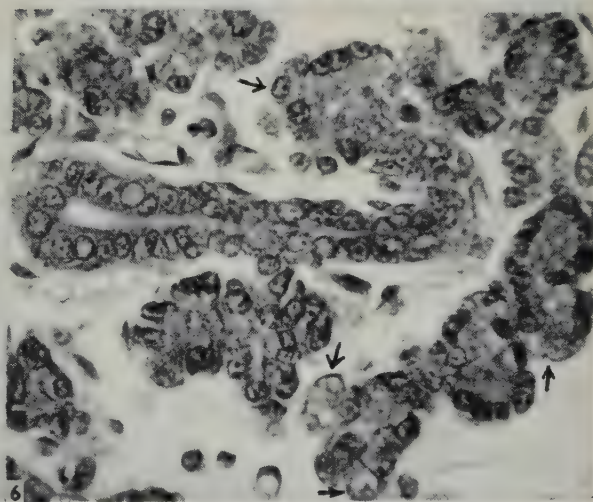
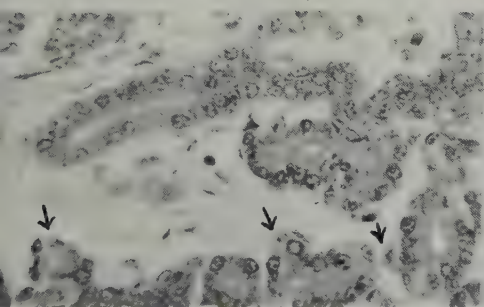
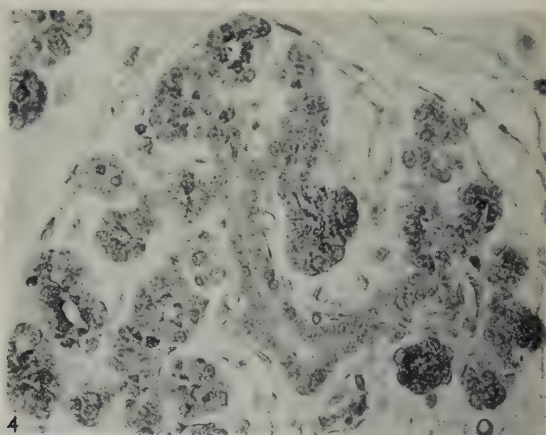
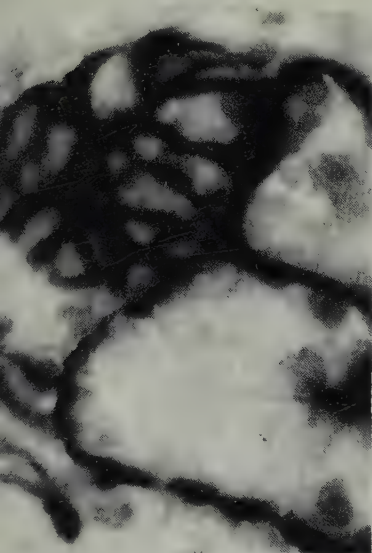
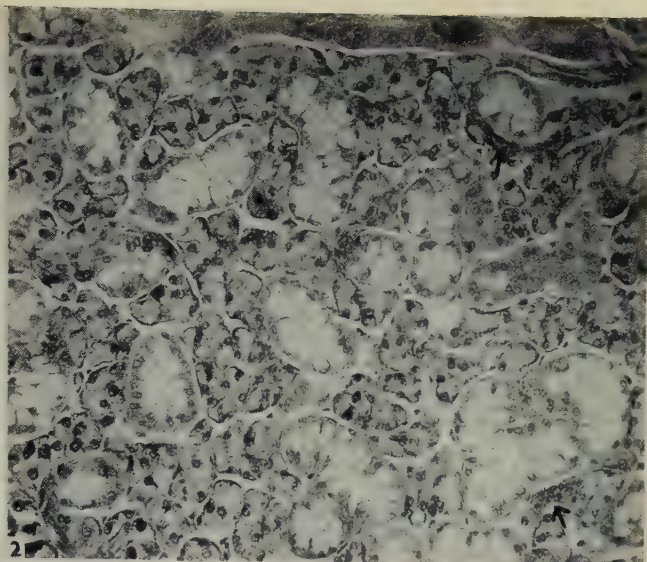
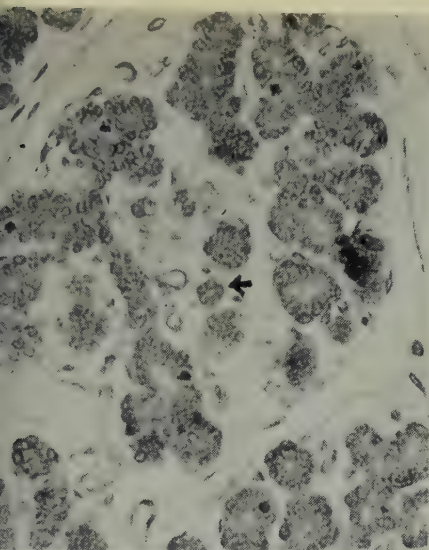
- Fig. 9. 3 weeks; fix. alc. Bouin, Hx. and Chr. A system is shown comprising intralobular and intercalated ducts, heavily granulated terminal tubules, which have become 'centres', and acinar crescents. Note the T-shaped junction between the ducts. $\times 710$.
- Fig. 10. 4 weeks; fix. alc. Bouin, Hx. and Chr. A system similar to that of Fig. 9 is shown. Note end-to-end junction of the ducts and the very deep staining of the granules of the centres. $\times 420$.
- Fig. 11 *a*. 5 weeks; fix. alc. Bouin, Hx. and Chr. Note convolutions of intralobular striated ducts. 'Centres' reduced in size and number. $\times 305$.
- Fig. 11 *b* (Inset). 5 weeks; fix. Susa, Hx. and eosin. T.S. of terminal tubule with crescents. In the very centre a nucleus of a 'centro-terminal tubular' cell. $\times 515$.
- Fig. 12. 6 weeks; fix. alc. Bouin, Hx. and Chr. Remnant of a centre in top right corner. An intralobular striated duct (L.S.) shows ill-defined junction with an intercalated duct. First signs (vacuolization) of transformation of some of the cells of the striated duct (\downarrow). $\times 365$.

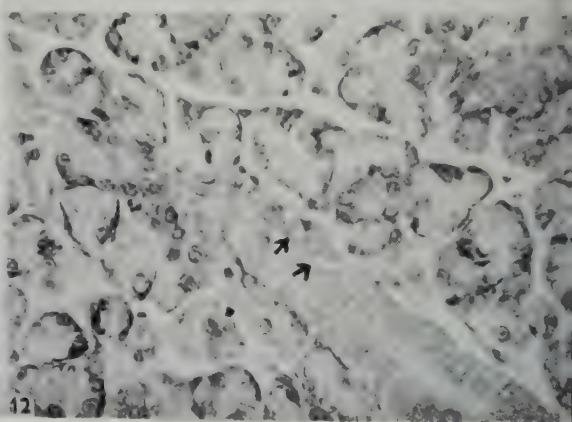
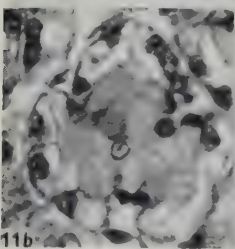
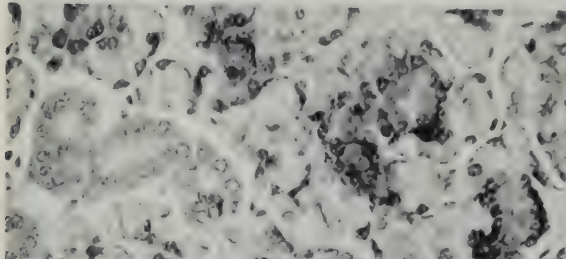
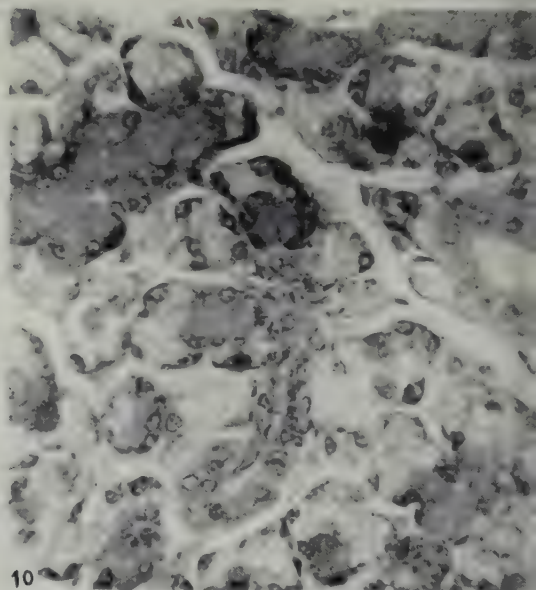
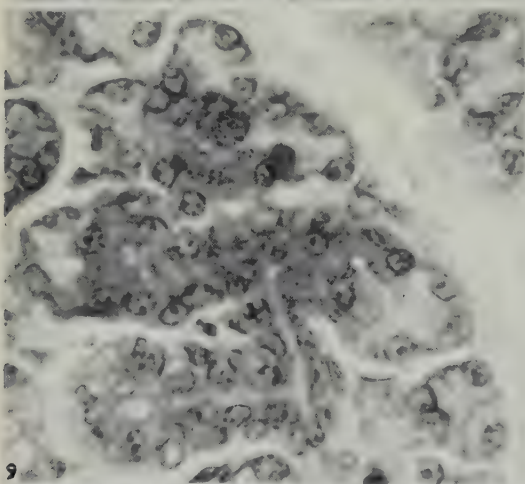
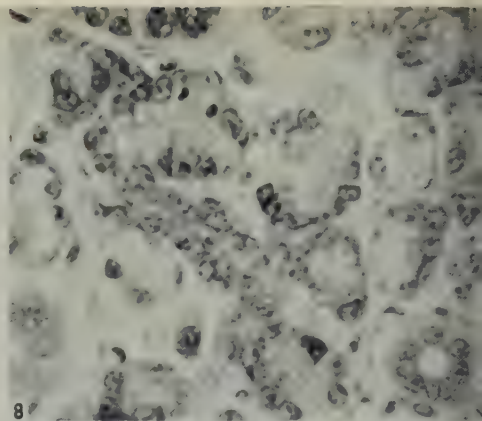
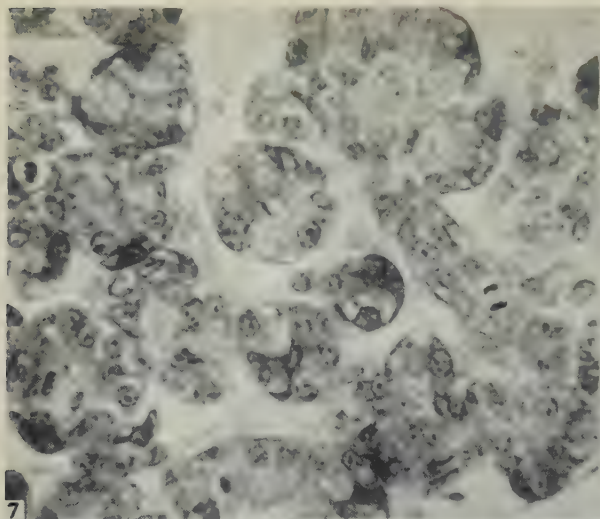
PLATE 3

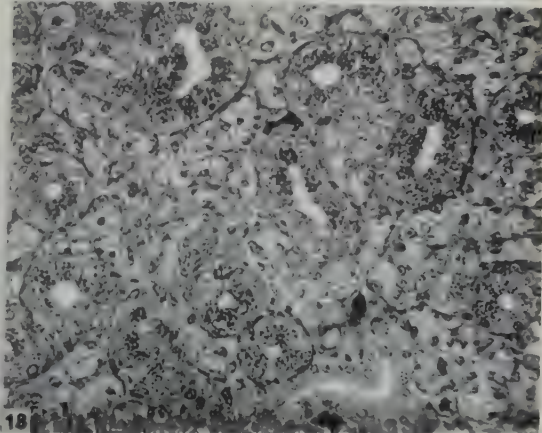
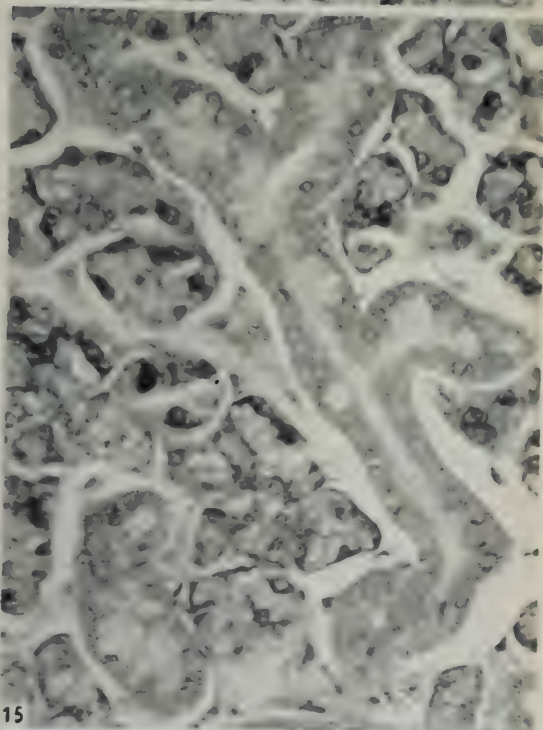
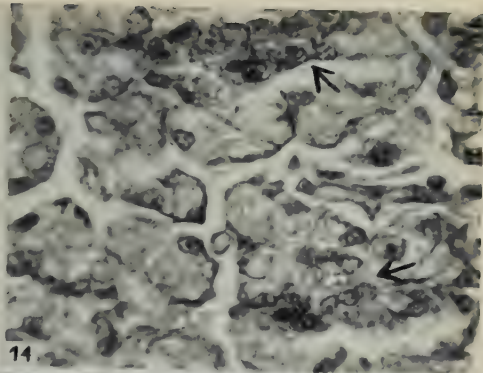
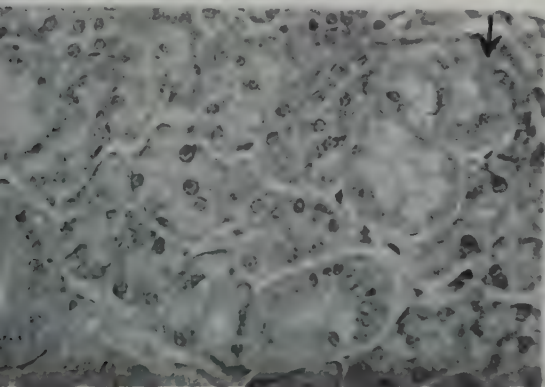
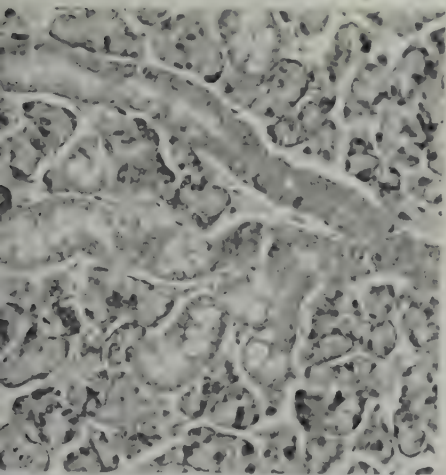
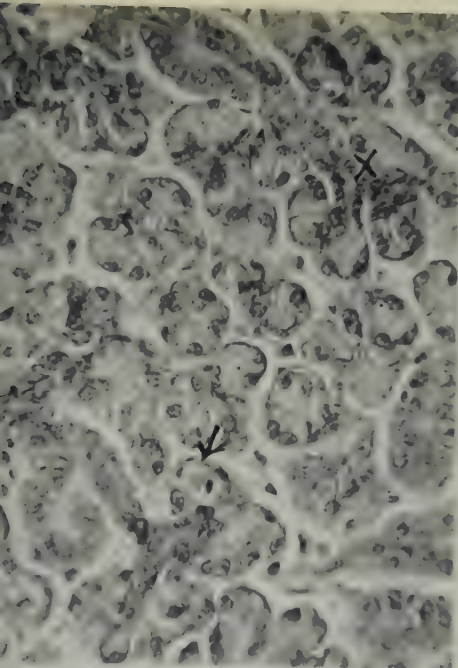
- Fig. 13. 6 weeks; fix. alc. Bouin, Hx. and Chr. Two systems can be made out; the upper one shows the reduction in size of the cells of the former centres, which have now become intercalated ducts, 2nd order (\times). Acinar mitosis arrowed. $\times 340$.
- Fig. 14. 6 weeks; fix. alc. Bouin, PAS reaction and Hx. Note two very much 'reduced' (narrowed) centres (\downarrow), the flat cells of which still contain PAS-positive granules. To the left, portion of an intralobular striated duct. $\times 515$.
- Fig. 15. 8 weeks; fix. alc. Bouin, Hx. and Chr., to show branching convoluted duct with more advanced transformation into 'granular' tubule. The change is clearly confined to the proximal segments. An ill-defined 'junction' in top left corner. $\times 320$.
- Fig. 16. 10 weeks; fix. alc. Bouin, Hx. and Chr. Further progress in transformation of the convoluted tubules. $\times 180$.
- Fig. 17. 12 weeks; fix. alc. Bouin, Hx. and Chr. Well-preserved basal striations in inter- and intralobular ducts. Fine granules can be seen in the transformed cells of the convoluted tubules. In top right corner a junction (\downarrow). Note here lack of transformation of the cells of the convoluted tubule. $\times 335$.
- Fig. 18. 16 weeks; fix. Susa, azan, to show the large amount of secretion granules in the cells of the convoluted granular tubules. Note also the amount of lobular space now occupied by granular tubules. In the lower part of the field, the transition from granular to non-granular intralobular duct can be seen. $\times 200$.

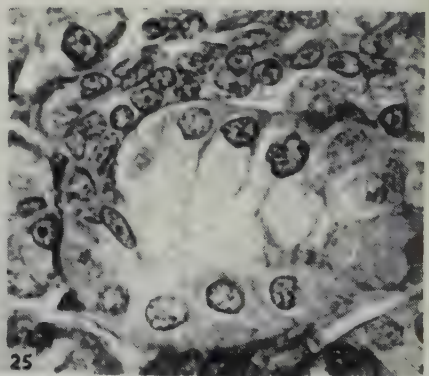
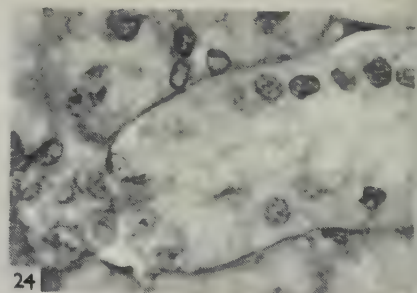
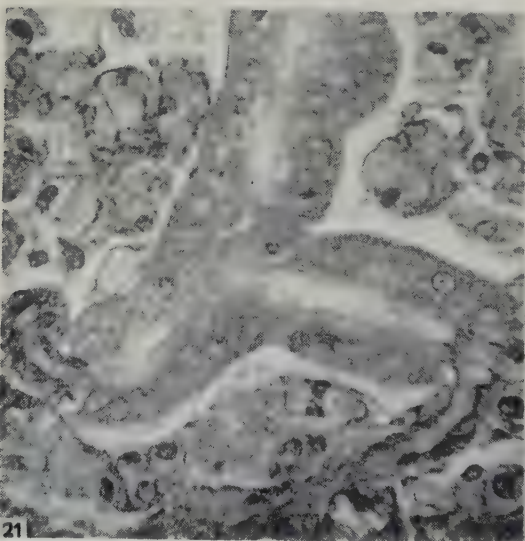
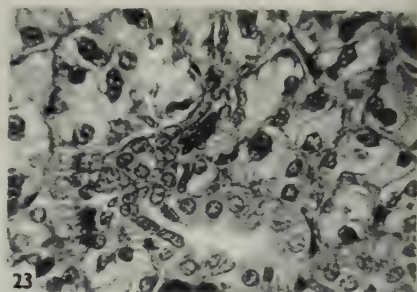
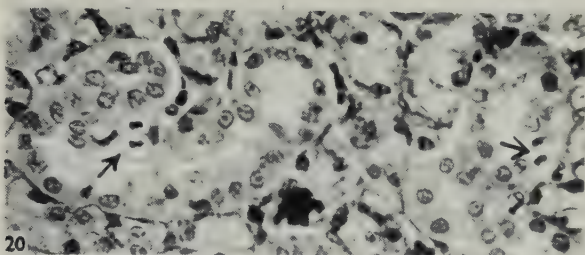
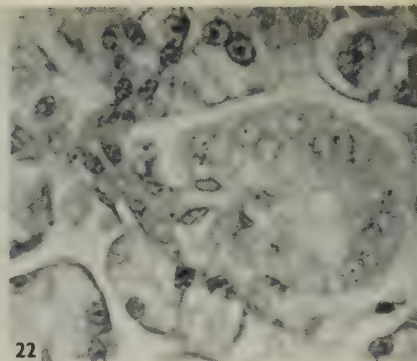
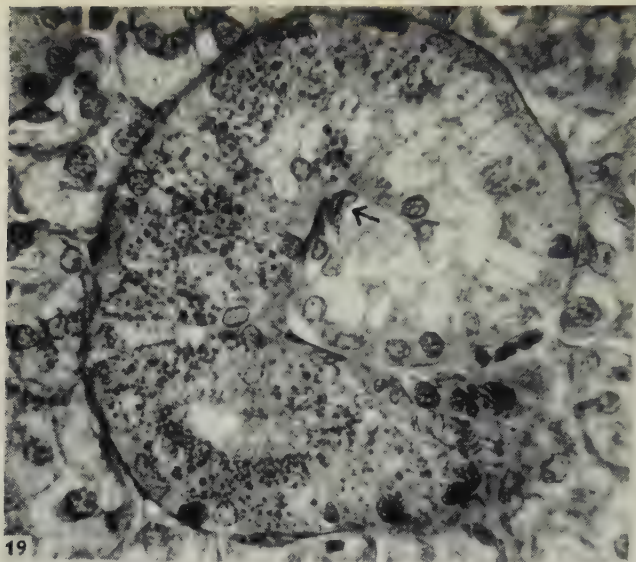
PLATE 4

- Fig. 19. 4 months; fix. Susa, azan. Most proximal portion of a convoluted granular tubule. Note variation in size of granules and in amount of granules in different cells, particularly the lack of granules in the coiled-in 'tip' of the tubule which just seems to show the 'junction' (\downarrow). $\times 515$.
- Fig. 20. 7 weeks; fix. Susa, azan, to show two mitoses (\downarrow) in convoluted tubules. All the pale cells of these tubules are in the process of transformation. $\times 355$.
- Fig. 21. 14 weeks; fix. alc. Bouin, Hx. and Chr. A branching striated duct is shown. At or near the 'pole' of each branch there is a junction with an intercalated duct. This specimen was not as fully developed as one would have expected from its age. $\times 490$.
- Figs. 22-25. Show various intercalated duct-striated duct junctions (Fig. 22, 8 weeks ($\times 515$), fix. alc. Bouin, Hx. and Chr.; Figs. 23, 10 weeks ($\times 355$), 24, 4 months ($\times 625$), and 25, 6 months ($\times 700$) fix. Susa, azan.). All show varying degrees of 'intussusception'; T-shaped junction is indicated in Figs. 22, 23 and possibly 24; the curved course of the intercalated duct around the convoluted granular tubule is well shown in Fig. 25. Note again scarcity of granulation in the cells of the very tip of the convoluted tubule in Figs. 24 and 25.









JACOBY AND LEESON—POST-NATAL DEVELOPMENT OF THE RAT SUBMAXILLARY GLAND

THE FINE STRUCTURE OF THE GASTRIC PARIETAL CELL IN THE MOUSE

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INTRODUCTION

The parietal cell which occurs in the stomach of higher vertebrates—including mammals—has been intensively studied since Heidenhain (1870) first distinguished it from the chief cell of the gastric glands, and suggested that it secreted hydrochloric acid.

Several workers, including Zimmerman (1898), by using Golgi's chrome silver method, demonstrated a system of intracellular canaliculi within the parietal cell which opened into the lumen of the gastric gland at the free surface of the cell. Fitzgerald (1910), with an intravital technique based on the Prussian blue reaction, confirmed the presence of these canaliculi in living mucosa, and Harvey & Bensley (1912) showed them particularly clearly with a supravital neutral red method. Others, including Beams & King (1932), reaffirmed their existence in both fixed and fresh mucosa, and recently Moussa & Khattab (1957) observed them in unstained fresh mucosa with phase contrast.

The characteristic oxyphil granules of the parietal cell were regarded by Altmann (1894) as mitochondria and this has been corroborated by later work. For example, Lim & Ma (1926) showed that the granules stained with acid fuchsin and Sudan black, while Menzies (1949) demonstrated that they contained phospholipine with a protein moiety. Moreover, the granules stained with Janus green B—recognized as a specific reaction of mitochondria—and which is, according to Lazarow & Cooperstein (1953), related to the presence of the oxidative enzymes localized within the mitochondria.

Ma, Lim & Liu (1927) consider the Golgi complex of the parietal cell to be merely lipid within the intracellular canaliculi, but most workers believe there is a Golgi complex distinct from the canaliculi. Thus, Moussa & Khattab (1957) describe a Golgi complex—with an affinity for osmium—as scattered thick filaments throughout the cytoplasm, or occasionally as a localized network.

The brief electron microscopical studies of Sedar (1955) and Challice, Bullivant & Scott (1957) describe the intracellular canaliculi.

In view, therefore, of its importance as the source of hydrochloric acid in gastric juice, and its unique system of intracellular canaliculi, the fine structure of the parietal cell was further studied with the electron microscope.

METHODS

Healthy adult mice, aged 6–9 months, were fasted for 24 hr., and then killed by a blow on the head. Small portions of mucosa were removed from the body of the stomach, and placed in fixative within 2 min. of death.

Processing

The specimens were fixed in isotonic buffered 1 % osmium tetroxide solution for 1 hr. After washing for 10 min. in normal saline, they were dehydrated rapidly through 70, 90 and 100 % ethyl alcohols. Methacrylate embedding was the same as in a previous communication (Hally, 1958) except that final polymerization was carried out at the higher temperature of 60° C. (Borysko, 1956). Sections were cut on a Cooke & Perkins thermal expansion microtome using glass knives (Latta & Hartmann, 1950) and mounted on collodion-coated copper grids.

Electron microscopy

A modified Philips EM. 100A was used in this investigation, but a few micrographs are included which were taken by the author on a Philips EM. 75B at Eindhoven.

OBSERVATIONS

In sections through the body of the gland, the chief or zymogenic cells contain—like other serozymogenic cells such as the exocrine pancreatic cell (Sjöstrand & Hanzon, 1954) or the Paneth cell (Hally, 1958)—secretory granules and a highly organized endoplasmic reticulum (Pl. 1, fig. 1).

The parietal cells—characterized by large dense ovoid mitochondria and a system of intracellular canaliculi—are readily distinguished from the mucous neck cell and the chief cell (Pl. 1, fig. 1). Often a parietal cell is interposed between two mucous neck or chief cells so that its apex reaches the lumen of the gland (Pl. 1, fig. 3). Alternatively, a parietal cell which does not reach the lumen of the gland directly, communicates with it via an intercellular canaliculus as in Pl. 1, fig. 2, where such a canaliculus leads from the narrow apex of a parietal cell towards the main lumen. The intercellular canaliculus is a narrow cleft between two adjacent cells, sparsely lined with short microvilli (Pl. 1, fig. 2).

THE PARIETAL CELL

The parietal cell is pyramidal, with a large basal surface next to the basement membrane and the apex towards the lumen of the gland. It is larger than the other cells of the gastric glands and has a centrally placed nucleus (Pl. 1, fig. 3).

In survey micrographs the ovoid, dense, large mitochondria are the dominant feature of the cytoplasm (Pl. 1, figs. 1–3). They appear to be separated into a peripheral group and a central perinuclear group by a ring-like lighter zone of cytoplasm containing the intracellular canaliculi (Pl. 1, fig. 3).

Higher magnifications reveal other cytoplasmic inclusions: a system of vacuoles adjacent to the intracellular canaliculi, vacuole-containing bodies (Pl. 2, fig. 5) (Rhodin & Dahlmann, 1956), Palade particles, and a scanty granular endoplasmic reticulum.

Intracellular canaliculi

Intracellular canaliculi—consisting of a branching system of tubular passages within the parietal cell opening on to the free apical surface to communicate with

the lumen of the gland either directly, or indirectly via an intracellular canaliculus—are seen in most sections.

The canaliculi—cut in cross-section—are clearly seen in Pl. 2, fig. 4, where they are more distended than usual. In some sections, canaliculi are cut longitudinally throughout the greater part of their course, and in one fortunate section (Pl. 1, fig. 3), two intracellular canaliculi can be seen extending from the basal part of the cell, upwards on both sides of the nucleus to reach the free surface of the cell, where they open directly into the lumen of the gland. In most micrographs the canaliculi appear as a complex system of membranes (Pl. 2, fig. 5), as their walls are not smooth, but have numerous microvilli projecting into and reducing the lumen.

The microvilli are bounded by a continuous cell membrane about 100 \AA thick, which occasionally has been resolved into a double membrane resembling that of the microvilli of the intestinal brush border (Zetterqvist, 1956) and the Paneth cell (Hally, 1958). There is, however, an additional membrane underlying this double membrane seen in both cross-sections and longitudinal sections of microvilli (Pl. 4, fig. 7; Pl. 5, fig. 8). This is a unique structural feature of the microvilli of the parietal cell.

The microvilli of the parietal cell have an average length of 0.7μ and a mean diameter of 0.09μ . The number of microvillous projections is about 10 per μ^2 —calculated by measuring the distance between the bases of adjacent longitudinally cut microvilli and assuming that the microvilli are regularly spaced. Direct measurement of cross-sectioned microvilli is unsuitable for the parietal cell as the microvilli converge on each other near the centre of the canaliculus. According to the above figures the microvilli of the parietal cell increase the secretory surface area threefold.

Cell membrane

Basally, where the cell membrane is related to a basement membrane it is infolded at infrequent intervals (Pl. 3, fig. 6). These folds are not comparable, however, to the highly complex system found in epithelia specially concerned with water transport (Pease, 1956).

Mitochondria

The great size and numbers of the oxyntic granules or mitochondria is the dominant feature of the parietal cell in survey micrographs (Pl. 1, figs. 1–3). Mostly ovoid, a few are in the form of short cylinders and the mean thickness—measured parallel to the cristae—is 0.575μ . This is greater than mitochondria of other cells: for example, the corresponding figure in the intestinal epithelium is 0.25μ (Zetterqvist, 1956) and in the renal tubular epithelium (Rhodin, 1954) is 0.4μ .

Their fine structure is typical of mitochondria in general, with an outer double membrane and internal cristae (Pl. 5, fig. 8). However, the ground substance of the parietal cell mitochondria is much denser than that of mitochondria in other cells (Pl. 1, fig. 2). The cristae show as two dense 75 \AA lines separated by a 40 \AA interspace, and are unusually well developed, extending across the full width of the mitochondria (Pl. 3, fig. 6; Pl. 5, fig. 8). Compared with mitochondrial cristae elsewhere, and even including those of such active cells as the renal tubular epithelium, the cristae within the parietal cell are more close-packed—about 30 to every micron length of mitochondrion.

Cytoplasmic vacuoles

Spherical vacuoles ranging in size from about $0.05\text{--}0.2\mu$ in section, pack the cytoplasm between the mitochondria and the intracellular canaliculi (Pl. 2, fig. 5; Pl. 5, fig. 8). Each vacuole is bounded by a smooth membrane, but although they are often contiguous, show no evidence of interconnexions, and so do not form a reticulum. A few vacuoles are also found more peripherally among the mitochondria (Pl. 3, fig. 6), but the majority lie adjacent to the canaliculi.

Endoplasmic reticulum

The granular endoplasmic reticulum appears as a few elongated profiles representing tubules, scattered throughout the cytoplasm (Pl. 3, fig. 6). The double nuclear membrane has a structure similar to that of other cells (Dawson, Hossack & Wyburn, 1955; Watson, 1955), and occasionally continuity between the outer nuclear membrane and these tubules of the endoplasmic reticulum is seen, as described in other cells by Watson (1955).

Palade particles, about $150\text{--}200\text{\AA}$ in size, are scattered in small groups of four to seven throughout the cytoplasm (Pl. 3, fig. 6).

Golgi complex

The Golgi complex has been identified with the electron microscope in a wide variety of cells since it was first described by Dalton & Felix (1954) and Sjöstrand & Hanzon (1954) as a system of smooth parallel membrane pairs, small granular vesicles, and large vacuoles.

Nothing conforming to this description has been found in the parietal cell, although a spurious resemblance to such a Golgi complex may arise where an intracellular canaliculus is cut transversely, and some of the microvilli—cut longitudinally—appear as smooth double membranes, while others—sectioned transversely—resemble Golgi vesicles.

It has not yet been possible to determine which cytoplasmic inclusion in the parietal cell corresponds to the 'Golgi complex' described under the light microscope, although preliminary work indicates it is neither the mitochondria nor the intracellular canaliculi.

DISCUSSION

*The intracellular canaliculi, mitochondria, and
their functional significance*

The intracellular canaliculi consist of a branching system of passages within the cell, lined by microvilli, opening at the free surface of the cell to communicate either directly or via an intercellular canaliculus with the lumen of the gland. Thus their general arrangement conforms with the light microscopical observations of Harvey & Bensley (1912).

What is the significance of this system of canaliculi? The parietal cell is approximately pyramidal, with the apex towards the lumen of the gland, so there is a large basal surface area for the exchange of materials with the underlying capillaries (Pl. 1, figs. 2, 3). On the other hand, the secretory surface, at the apex of the cell,

would be small were it not greatly augmented by the intracellular canaliculi, which can be regarded as invaginations of the secretory surface. Assuming that the total length of canaliculi within a cell is 50μ —probably an underestimate as in a single section (Pl. 1, fig. 3), as much as 40μ can be seen—and as the average diameter is about 1μ , this would give an additional surface area of $160\mu^2$ if the walls of the canaliculus were smooth. The microvilli, however, will further increase this surface by a factor of three, giving a final figure of about $500\mu^2$, which compares favourably with the available secretory surface area of other cells. Finally, the presence of the canaliculi means that secretion formed anywhere within the cell has to be transported only a short distance to reach the secretory surface, and so pass into the lumen of the gland.

Therefore, the intracellular canalicular system within the parietal cell enables it to be compact, while retaining extensive basal and secretory surfaces, and ensuring that secretion formed within the cell has ready access to the lumen of the gland—factors which will increase the efficiency of a secretory cell.

A unique feature of the microvillus of the parietal cell reported here, is an additional membrane underlying the bounding cell membrane, which distinguishes it from microvilli of other cells (Rhodin, 1954; Yamada, 1955; Zetterqvist, 1956; Hally, 1958). The significance of this membrane is unknown.

Abundant large ovoid mitochondria, with numerous cristae and dense ground substance are a feature of the parietal cell. There is convincing biochemical evidence that mitochondria are concerned with almost all oxidative processes involving the consumption of oxygen, and recently Green, Lester & Ziegler (1957) have shown that structurally intact cristae are necessary for oxidative phosphorylation—the energy-producing mechanism of the cell. The number and size of the mitochondria, with their unusually extensive and close-packed cristae, will give the parietal cell a total area of cristae of a high order. Therefore, the morphological implications are that the actively secreting parietal cell has a high rate of oxygen consumption.

Although it is generally agreed that the parietal cell secretes the hydrochloric acid of gastric juice, the exact nature of the metabolic mechanism and the form in which it is secreted are unknown. Any theory of the mechanism of hydrochloric acid secretion, however, must consider the efficiency of the process, i.e. the ratio of acid secreted to oxygen consumed (Davies, 1957). This ratio, according to most workers including Davenport (1957), is believed to be less than 4. This figure, however, implies that the parietal cell has a greater oxygen consumption rate than that of any other mammalian cell, and as Davies (1957) considers this unlikely, he concludes that any mechanism which entails a ratio of only 4 is untenable.

The fine structure of the parietal cell, however, and in particular the unique system of intracellular canaliculi and very abundant mitochondria, indicates a specialized efficient cell with an unusually high oxygen consumption rate, and so gives some morphological support to the views of Davenport (1957).

The endoplasmic reticulum and cytoplasmic vacuoles

The examination *in toto* of spread cells led to the introduction of the term 'endoplasmic reticulum' to describe a system of interconnected vesicle-like bodies lying in the cytoplasm. Since then it has been identified in every type of cell examined,

except the mature erythrocyte (Palade, 1956). In ultra-thin sections, according to Palade (1956) 'the reticular nature is lost' as the likelihood of seeing the interconnecting strands is much reduced owing to the thinness of the sections, and so the endoplasmic reticulum appears to consist of unconnected sacs, tubules, or vesicles. Structurally, the endoplasmic reticulum has two forms—the agranular reticulum bounded by smooth membranes, and the granular reticulum bounded by membranes studded on one surface with Palade particles or granules. The relative volume, shape, and distribution of those two forms of reticulum varies widely in the different types of cell, and Palade (1956) classifies types of endoplasmic reticulum ranging from the highly organized granular reticulum of the plasma cell to the mainly smooth agranular reticulum found in such cells as the rat spermatocyte—which he describes 'as characterized by a randomly disposed, predominantly smooth surfaced reticulum made up primarily of interconnected vesicles and tubules'. He further states that such a reticulum occurs in certain other cells, and quoting the work of Sedar (1956) includes the parietal cell in this group. According to Sedar (1956) the cytoplasmic vacuoles adjacent to the canaliculi constitute the smooth agranular reticulum of the parietal cell, but these vacuoles do not conform to the above description, as they are not randomly disposed, but are concentrated around the canaliculi, nor are they interconnected—although being close-packed and small, random sections would be expected to show such interconnections if they did exist.

Thus the present observations suggest that the cytoplasmic vacuoles within the parietal cell cannot be regarded as forming a reticulum, and differ fundamentally from the smooth-surfaced endoplasmic reticulum of such cells as the rat spermatocyte.

Challice *et al.* (1957) consider the possibility that these cytoplasmic vacuoles represent cross-sectioned microvilli, but it is unlikely that large numbers of microvilli would be consistently cut transversely, and the unique structure of the microvilli, with the additional underlying membrane, enables the two structures to be readily distinguished (Pl. 4, fig. 7; Pl. 5, fig. 8).

SUMMARY

1. The gastric parietal cells of the mouse were examined electron microscopically using osmium tetroxide fixation.
2. A system of branching intracellular canaliculi lies within the parietal cell, and open at the free surface of the cell into the lumen of the gland, either directly or via an intracellular canaliculus. The numerous microvilli lining the canaliculus have a unique structure, an additional membrane underlying the bounding double cell membrane.
3. The mitochondria, which are abundant, large, and ovoid, are characterized by a very dense ground substance and exceptionally well-developed cristae. These features are considered to be related to the high metabolic rate of the parietal cell.
4. Cytoplasmic vacuoles are found mainly in relation to the intracellular canaliculi. They are independent spherical vacuoles which do not form a reticulum.
5. No typical Golgi complex exists within the parietal cell.
6. The functional significance of the system of intracellular canaliculi is discussed.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Section through the body of a gastric gland. A peripherally placed parietal cell (*P.*) is adjacent to several chief zymogenic cells, which are characterized by a highly organized granular endoplasmic reticulum (*E.R.*) and secretory granules (*S.*). $\times 2700$.
- Fig. 2. A parietal cell with very large dense mitochondria (*M.*), a centrally placed nucleus (*N.*), and an intracellular canaliculus (*C.*) which opens on the free surface of the cell to become continuous with a narrow cleft between adjacent mucous neck cells—an intercellular canaliculus (*I.C.*). $\times 3200$.
- Fig. 3. A micrograph showing a parietal cell interposed between mucous neck cells so that its free surface bounds part of the main lumen (*L.*) of the gland. Basal to the nucleus (*N.*) of the parietal cell are two intracellular canaliculi (*C.*) which extend past the nucleus to reach the free surface of the cell and open directly into the lumen of the gland (arrows). Two capillaries (*Cap.*) are subjacent to the parietal cell. $\times 5700$.

PLATE 2

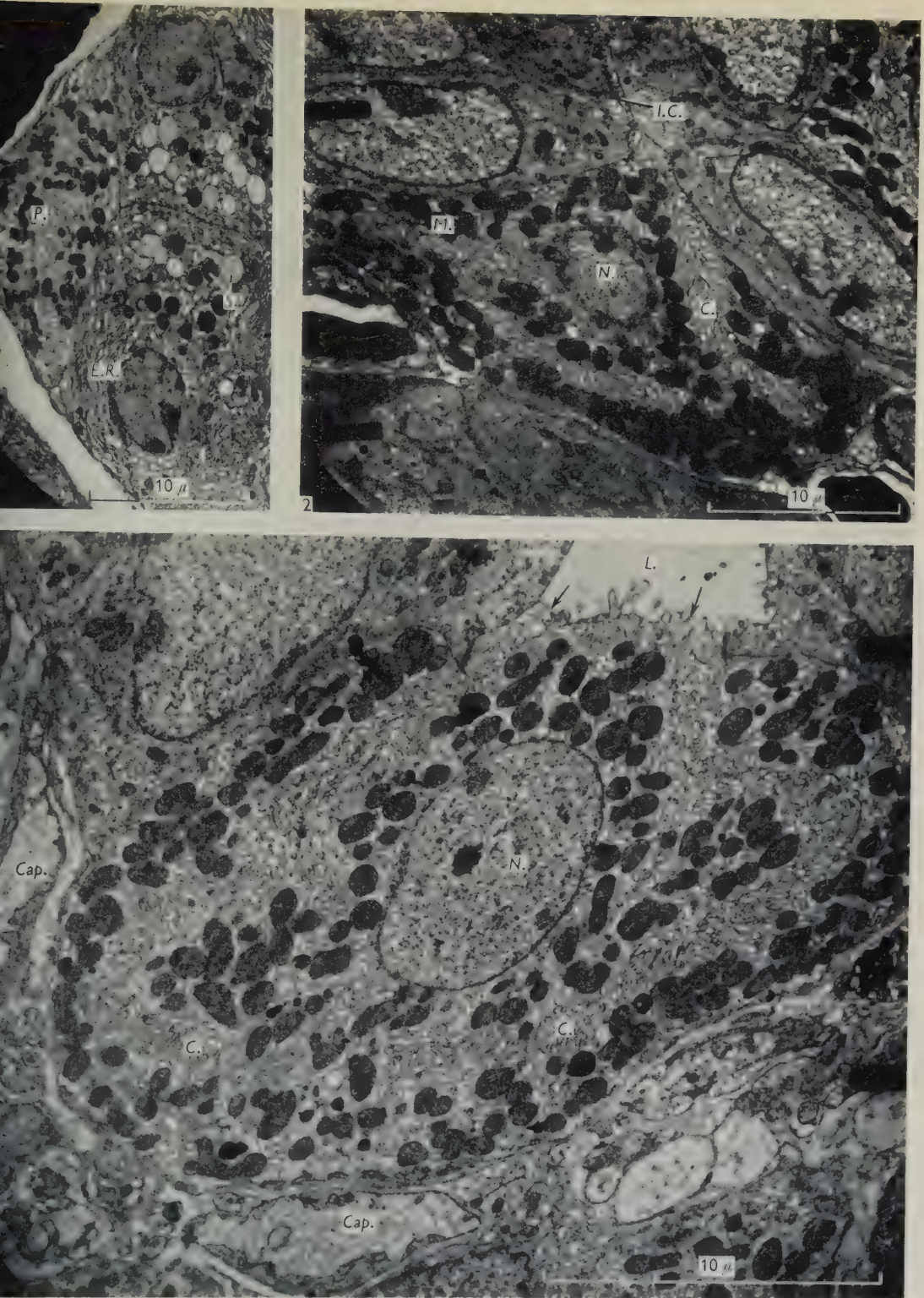
- Fig. 4. Within the parietal cell occupying the left half of this micrograph are several cross-sectioned intracellular canaliculi (*C.*). Microvilli project into the lumen of each canaliculus, rendering its outline irregular. $\times 5200$.
- Fig. 5. Part of a parietal cell containing large ovoid mitochondria (*M.*) with close-packed cristae and dense ground substance. An intracellular canaliculus extends from the upper right corner (*C₁*) obliquely downwards to the lower left corner (*C₂*). The lumen of the canaliculus is practically filled with microvilli (*MV.*). Many spherical cytoplasmic vacuoles (*V.*) lie between the canaliculi and the mitochondria. A single vacuole-containing body (*V.C.B.*) is also present. $\times 24,000$.

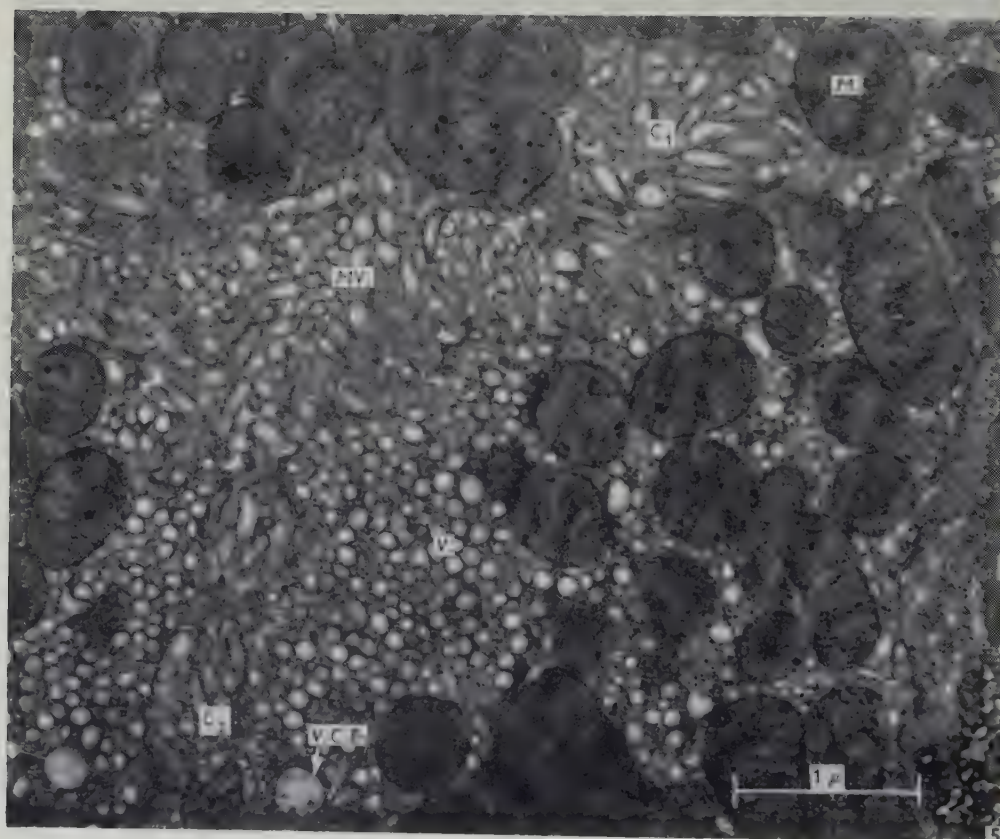
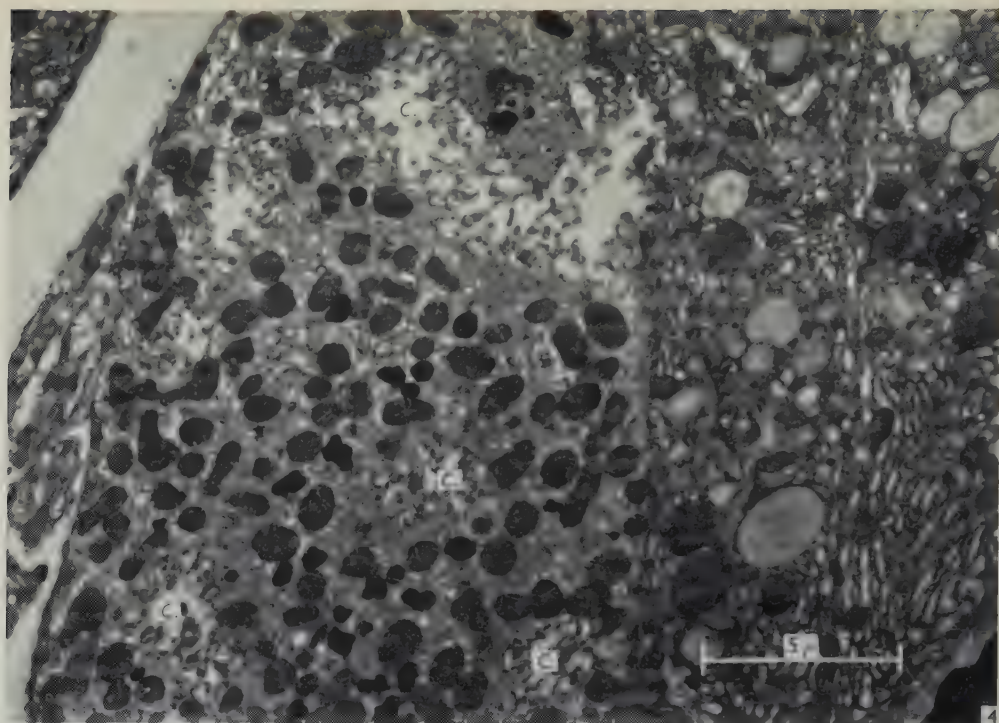
PLATE 3

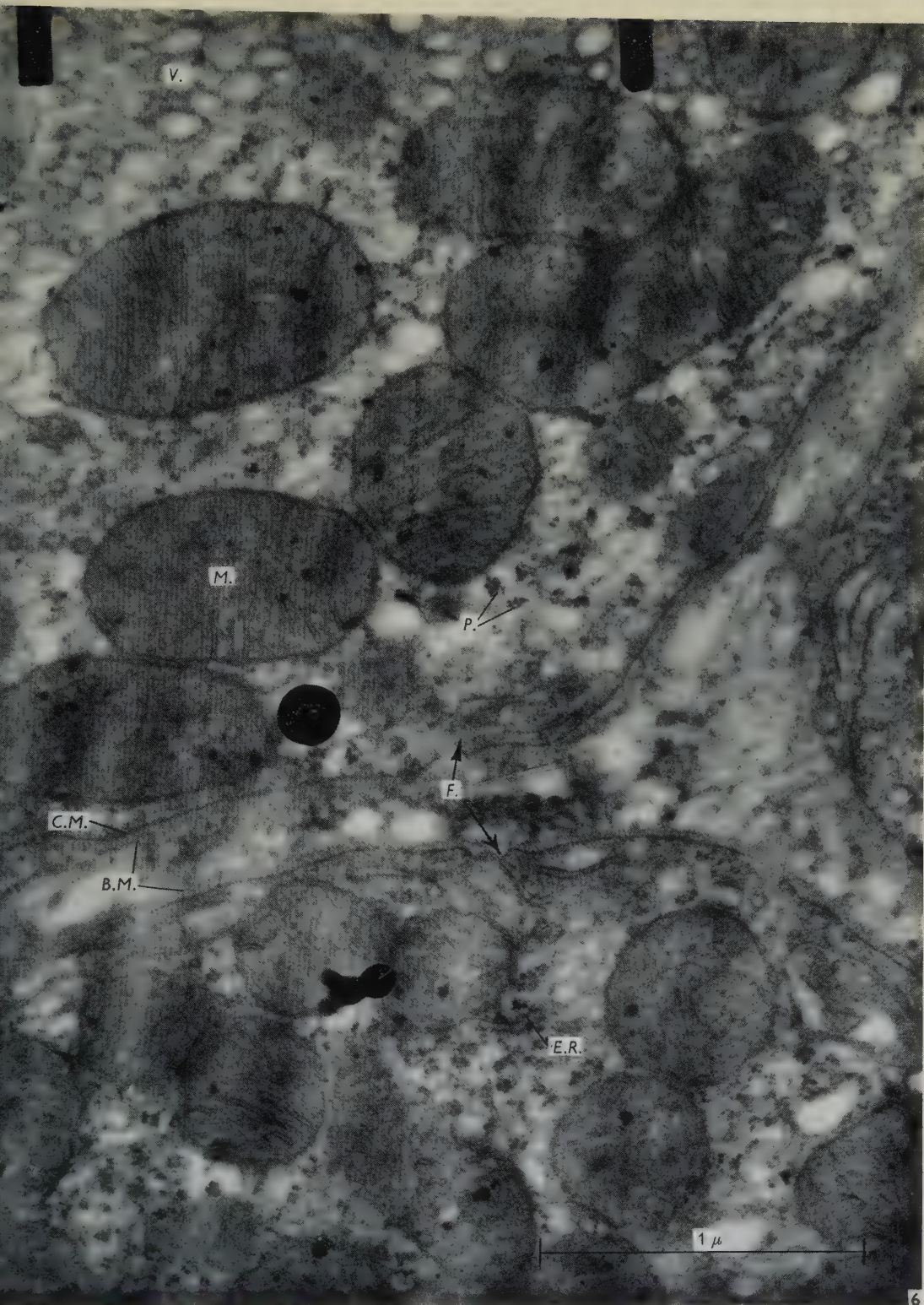
- Fig. 6. A micrograph of the basal portions of two adjacent parietal cells. The cristae show clearly within the mitochondria (*M.*) as double membranes. The cell membrane (*C.M.*), except where it is infolded (*F.*) into the cell, is in close relation to the underlying basement membrane (*B.M.*). Small clusters of Palade particles (*P.*) lie free in the cytoplasm and there are a few profiles of granular endoplasmic reticulum (*E.R.*). There is a paucity of cytoplasmic vacuoles in the peripheral cytoplasm near the cell membrane compared to the large numbers (*V.*) found in the uppermost part of the micrograph nearer the intracellular canaliculi. $\times 50,000$.

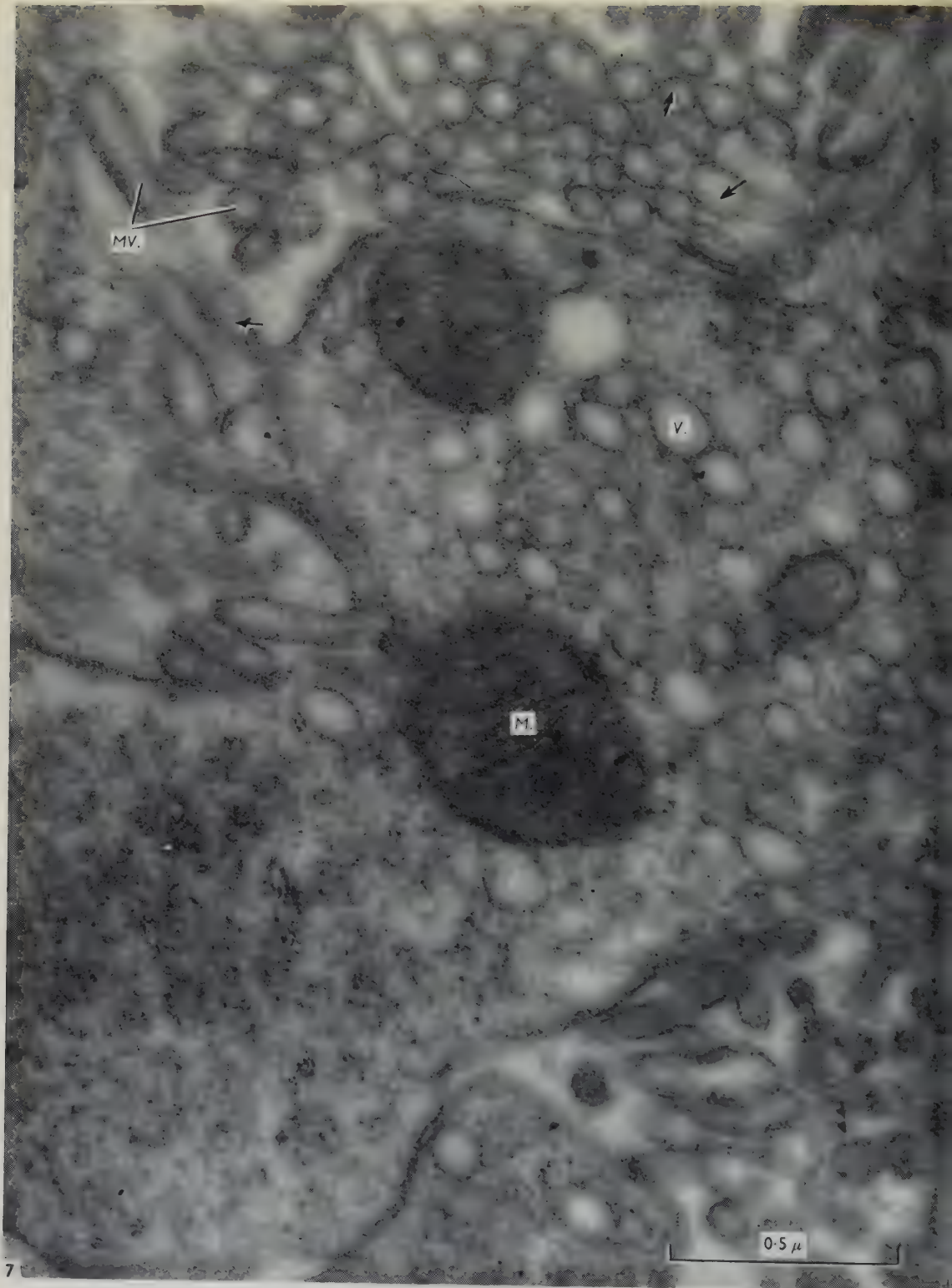
PLATE 4

- Fig. 7. (Philips EM.75B). A micrograph of the apical portion of a parietal cell. The intracellular canaliculus in the upper part of this micrograph contains numerous circular profiles of cross-sectioned microvilli and other microvilli cut longitudinally, an example of each being indicated (*MV.*). In addition to the cell membrane bounding the microvillus, there is an underlying membrane, visible in both cross-sectioned and longitudinally sectioned microvilli (arrows). Cytoplasmic vacuoles (*V.*)—each bounded by a single smooth membrane—and mitochondria (*M.*), are also seen. $\times 72,000$.









HALLY—GASTRIC PARIETAL CELL IN THE MOUSE

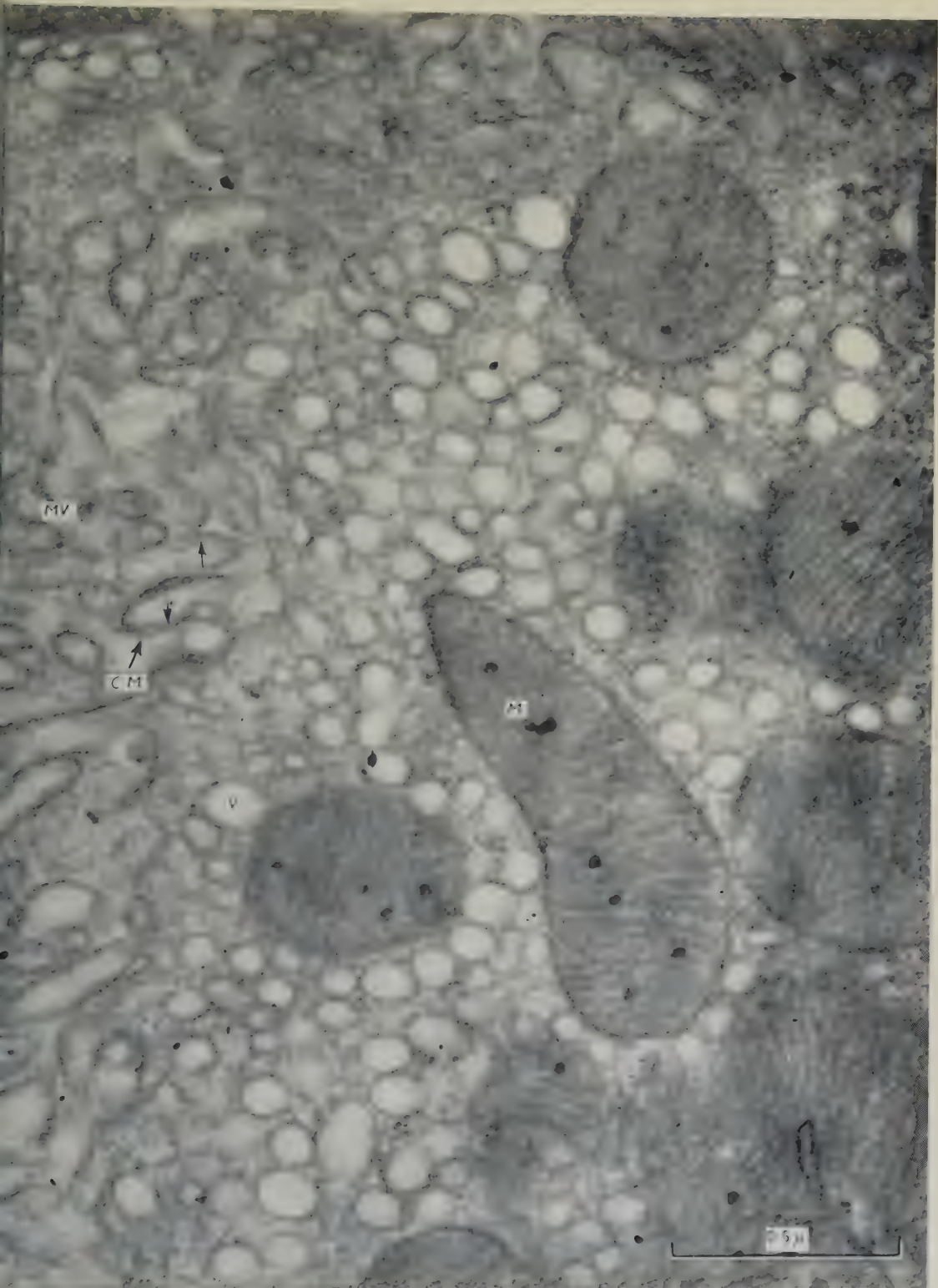


PLATE 5

Fig. 8. (Philips EM.75 B). An intracellular canaliculus—lined with inwardly projecting microvilli (MV.)—lies close to the left margin of this micrograph of a parietal cell. Most of the microvilli are cut longitudinally or obliquely, and some show the characteristic additional membrane (arrows) underlying the cell membrane. Cytoplasmic vacuoles (V.) pack the cytoplasm between the intracellular canaliculus and the mitochondria. Each spherical vacuole is bounded by a smooth single membrane, and is unconnected with neighbouring vacuoles. $\times 72,000$.

AN ELECTRON MICROSCOPIC STUDY OF THE HUMAN AXILLARY APOCRINE GLAND

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INTRODUCTION

According to Kuno (1956) sweat glands were discovered by Purkinje in 1833, and described by his pupil Wendt. Almost simultaneously they were described in 1834 by Breschet and Roussel de Vouzeme.

Montagna (1956) states that it was Krause (1844) who first observed that the sweat glands in the axilla and a few other sites differed from the commoner sweat gland by being larger, while Rothman (1954), quoting an editorial in the *British Medical Journal* (1939), says that the axillary glands were first described by Horner in 1846.

In 1887 Ranvier (see Kuno, 1956) differentiated the 'holocrine' secretion of the sebaceous gland from the 'merocrine' secretion of the sweat glands, and Schieferdecker (1922) further subdivided the mode of secretion of the merocrine glands into eccrine, and epicrine or apocrine, so naming the gland with which this paper is concerned.

Three decades later the work of Hurley & Shelley (1954) helped considerably to clarify our knowledge of the functioning of the gland.

In this paper a description will be given of the secretory and ductal parts of the gland as seen in the electron microscope.

MATERIAL AND METHODS

Axillary skin was obtained from a 34-year female under general anaesthesia for excision of an axillary abcess. The axilla was swollen by the volume of pus, but the abcess seemed well contained and the overlying skin was quite normal in appearance.

Fixation, embedding, and sectioning was in the manner described in a previous paper, Charles & Smiddy (1957), fixation commencing about 10 min. after the first incision.

The sections were examined on carbon-filmed grids in a Metropolitan Vickers EM3 electron microscope.

RESULTS

Pl. 1, fig. 1 shows part of a section of the secretory region of the apocrine gland. A single layer of columnar secretory cells of various lengths project into the lumen. The cells are based on myoepithelial cells whose outer wall forms the limiting membrane between the gland and the dermis.

The secretory cells contain dark secretory granules (*gr.*) and lighter mitochondria in which, on close examination, very unusually arranged cristae can be detected.

Nuclei are present in the majority of cells, but no nucleoli are sectioned. The Golgi apparatus can be observed in four of the secretory cells, especially in the one marked (*g.*), and in its vicinity a great number of tiny vesicles are seen. These vesicles, as will be shown clearly later, accumulate at the cell apices.

Canaliculi run between the upper parts of adjacent cells, as can be seen more clearly in Pl. 1, fig. 2, an oblique transverse section. This contradicts Kuno (1956, p. 46) who believes that canaliculi are absent. Cell membranes form the delicate protuberances, or papillae, projecting into the canaliculi.

On the two left-hand cells the membrane is papillate at the apex, the two next cells show a smooth apical membrane, and in the two right-hand cells the apical membrane has ruptured and a loss of vesiculate material has left an underlying clear space, the so-called hyalin cytoplasm (Montagna, 1956). The membranous ring (*i.s.*) in the lumen on the left of Plate 1, fig. 1 appears to be a transverse section through this clear region of the cytoplasm of a cell. The walls between adjacent cells are devoid of tonofibrillar prickles.

The Golgi apparatus and secretory granules are more clearly seen in Pl. 2, fig. 3. Located among the granules and close to the nucleus it appears to be giving rise to the innumerable tiny vesicles which are confined to its vicinity.

The secretory granules are of two kinds, 'smooth' (*s.g.*) and 'rough' (*r.g.*). The former can be differentiated from the mitochondria only by the absence of cristae, whereas the rough granules show a peripheral granulation into small dense particles which appear collectively as a dark margin on the granule. In other sections the granule may appear as a large, apparently hollow, oval structure with a shell of dense, various-sized droplets or granules; or consist of a lighter 'kernel' embedded in dense, finely particulate material.

The myoepithelial cells are much more strongly developed in the apocrine gland than in the eccrine. When longitudinally sectioned they are 60μ or more in length, containing long dark fibrils embedded in a lighter inhomogeneous looking material (Pl. 1, fig. 1), which fibrils appear in transverse section as dots (Pl. 2, fig. 4). Between the cells, and covering also those parts of the myoepithelium in contact with the dermis (Pl. 3, fig. 4), there is a dark, homogeneous, material assumed to be an extensible cementing substance. Occasionally the secretory cells project downwards between the myoepithelial cells and make direct contact with the dermis (Pl. 2, fig. 5). At their base the secretory cells have a much infolded basal membrane which is seen to be double when transversely sectioned (Pl. 2, fig. 5). Between the myoepithelium and the secretory cells intercellular spaces are commonly found.

Pl. 3, fig. 6 illustrates the appearance of the wall of the duct of the apocrine gland. In this section it is about three cells thick, the outer cells, at the bottom of the figure, being next to the dermis and the inner ones line the lumen of the duct. The outer limiting membrane is covered with a fine network of fibrous material, reticulin, while the surfaces of the inner cells are papillate. Embedded in the wall is a large clearer structure assumed to be a non-medullated nerve fibre. The cells of the duct are clearly different from the secretory cells. They are comparatively rich in mitochondria, which are generally much smaller than those found in secretory cells, but secretory granules and vesicles are absent. Golgi structures have not so far been observed. A well-developed prickle system is seen which suggests that the

duct is quite robustly constructed; there is much tonofibrillar material in the cytoplasm.

The papillae are simple structures, which consist of an outer denser membrane enclosing a homogeneous inner cytoplasm; they are mere protuberances of the cell surface.

Under higher magnification the unmyelinated nerve fibre appears very similar to Robertis & Ferreira's (1957) electron micrograph of an unstimulated nerve-ending in the adrenal medulla of the rabbit. There is a clear cytoplasm, bounded by the synaptic membrane, containing a number of mitochondria and many synaptic vesicles. Also present are granules showing internal structure which do not appear to be mitochondria, and a single dense granule showing no internal structure.

Pl. 4, fig. 7 shows a general view of the opening of the duct into the hair follicle. The opening is very irregular and multiple, many more smaller openings (*s.o.*) being observable if the area covered by the figure were increased. The surfaces bordering the lumen are richly papillate. Individual cells are sharply outlined by a very strongly developed prickly system, and contain much tonofibrillar material. This suggests that, although in this region they are much frayed by the channellings of the duct opening, the cells are in fact more robust than might at first be expected.

An interesting point here is that, for the first time, we see in the lumen some evidence of the products of the secretory cells. A fair amount of vesiculate material can be seen, together with what is presumably cell debris. These facts are better appreciated by looking at Pl. 4, fig. 8, which is an enlargement from the bottom right corner of Pl. 4, fig. 7. Here the vesicles are clearly seen and are obviously different from the transversely sectioned papillae. They are, indeed, generally smaller than the vesicles observed in the apices of the secretory cells, being about the same size as those in the region of the Golgi structure.

The prickly system, with intervening double cell walls, is well shown in the figure. Also to be observed are the intracellular canals (*ic.c.*) which convert the cytoplasm of cells bordering the lumen into a veritable sieve. These canals tend to disappear in cells away from the lumen; their like has been observed also in the ductal cells of the eccrine gland (Charles, unpublished).

DISCUSSION

So far as the present structural investigation goes it seems to support what Rothman (1954) calls the 'time-honoured view' of apocrine secretion—that the gland both secretes simply through the membranes of its cells and necrobiotically by a presumed exudation of cell contents after apical breakdown of the membrane. The evidence for simple secretion is the presence of canaliculi between the secretory cells, and the protrusion of the bordering cell surfaces into delicate papillae, so often associated with secretory tissue, whose purpose is presumably to increase surface area. The membranes bordering the canaliculi are invariably intact, and, while it is difficult to understand the function of these canals if the gland secretes only by apical breakdown of the cell, it is easy to understand their function if there is, additionally, secretion through the cell walls. The more robust and numerous papillae of the non-secretory duct, especially at its opening in the hair follicle, may be concerned with altering the composition of the original secretion before its final

exudation as sweat, much as the kidney tubules control the composition of the glomerular filtrate before its passage as urine (Rothman, 1954, p. 190). The relatively large number of mitochondria found in these cells, and the innervation of the duct tend to support such a postulated function. It is most unlikely that the papillae function as cilia to sweep out the secretions because motile cilia, contrasting with the simple papillae, have a complex electron microscopic structure, see Grigg & Hodge (1949), or Fawcett & Porter (1954).

The apocrine function of gland cells is puzzling in so far as no cellular exudate or debris has been observed in the lumen of the secretory part of the gland. This fact is mentioned by Montagna (1956, p. 166), and it would doubtless be instructive to section a dilated tubule, or non-osmidrotic gland (Kuno, 1956, p. 49), both of which are said to contain abundant luminal debris.

There is certainly a loss of apical contents when the membrane breaks down, but the loss seems to consist only of a proportion of the innumerable small vesiculate structures observable in the cell cytoplasm; other granules, mitochondria, and the residuum of the vesicles draw themselves closely about the nucleus and remain in the ruptured cell, whose apical membrane withdraws inwards in response to the slight decrease of volume. The presence of these vesicles in the secretion has been observed only at the orifice of the duct (Pl. 4, fig. 7) where there also occurred a structure which may, as equally it may not, have been a mitochondrial remnant, or a secretory granule. The size of the vesicles suggests that they have become more finely dispersed during their upward passage; their presence in the sweat could well give it its milky appearance. It is possible, however, to question whether the material at the orifice has not come down, rather than up, the duct, and is of foreign origin.

The observations in this paper are confined to one lot of material, and as such lack the advantages to be gained from observing individual and developmental variations. Differences between 'broken-down' and intact cells have not been great. It is unnecessary for cells to elongate greatly before rupture because short plump cells have been observed with ruptured apices; this, together with the apparent absence of severe collapse of apocrinely secreting cells, agrees with Kuno's (1956, p. 49) contention that the variation of size in the cells is inherent, and not due to their being observed at different stages of secretory activity.

It seems quite clear that apocrine secretion by the cells is not an especially well co-ordinated process; indeed it appears rather haphazard, ruptured cells mingling with intact. Because of this any clear eccrine-type secretion from the unruptured cells would always be mixed with milky apocrine secretion, so it would normally be difficult for the apocrine gland to exude a clear sweat. The presence of canaliculi, however, supports the possibility of eccrine-type secretion, and is consistent with the transitional eccrine functioning of the apocrine during infancy (Kuno, 1956, p. 52). Holmgren (1922) and Ota (1950), cited in Montagna (1956, p. 165), postulate eccrine cells in the apocrine gland. Functionally it seems likely that their belief is correct, but structurally different cells have not been observed.

The apical breakdown of the apocrine cells appears to be a slow eroding process, rather than a dramatic one brought about by internal cell pressure. Nevertheless, a sudden contraction of the myoepithelium must surely cause such internal pressures,

and cannot be without effect on the secretion of the cell during the later stages of membrane breakdown. If we except this mechanical effect, however, apocrine secretion would appear to depend on the ageing of the cells, possibly under hormonal control (Hurley & Shelley, 1954), but relatively or completely unresponsive to sudden stimuli. The eccrine-like function may, on the other hand, be more responsive to such stimuli, and it is not inconceivable that under certain conditions an apocrine gland may function very much in the manner of an eccrine.

It is likely that the vesiculate structures which leave the cell when it ruptures are formed in the Golgi apparatus. This apparatus is commonly found in the apocrine cells, and occupies a position just above the nucleus, between it and the cell apex. As shown in Pl. 2, fig. 3 the vesicles in this region are considerably more numerous than in the rest of the cytoplasm, and of a somewhat smaller size.

Speculation on the origin of the other secretory particles is even less certain. They are of a size similar to the mitochondria, and the so-called smooth granules differ from the mitochondria only in the absence of the characteristic cristae. For the rough granules there is some indication of development stages, because they have the appearance of being derived from an originally smooth granule by granulation of the surface into small dense particles, which gradually spreads inwards to the centre. Whether in fact the smooth granule is a development stage of the rough granule, or whether both granules are completely different entities, cannot be said. The nature of the small dense particles formed during granulation is also unknown; they may be lipid and they could equally well be pigment.

It is difficult to understand why secretory granules are so rarely, if at all, found in the lumen. Difficulty is experienced also with the secretory granules in the cells of the eccrine gland (Charles, unpublished) where there is no support at all electron microscopically for Ito's postulate (cited in Kuno, 1956, p. 52) of apocrine secretion by minute protuberances of the inner cells of the secretory part of the gland. In this case there seems no way whatsoever by which the granules can escape, whereas in the apocrine gland there is a way but it does not seem to be used. Whether we should consider these granules as reserve depots of material to be broken down as required and secreted in a soluble (eccrine) or emulsified (apocrine) form, or whether the granules are by-products of cellular metabolism, is something which needs further investigation. The present position of our knowledge is most unsatisfactory, and I feel a more dynamic study of the granules will have to be forthcoming before we can make any appreciable advance.

The mitochondria show a wide variation of shape and size, and while the arrangement of cristae on some of them is relatively conventional, the arrangement on the others is odd. In these, cristae-regions project as cristae-blisters giving the mitochondrion an unusual outline. Since the secretory granules are always smooth in outline this may indicate that they do not arise by transformation of the mitochondria.

The complex infolding of the basal membrane of the secretory cell is presumably designed to increase the surface area in contact with nutrient fluids from the dermis. Intercellular spaces seen between the myoepithelial cell and the base of the secretory cell may be artefacts; they may also arise naturally, because the myoepithelium will almost certainly pucker the secretory layers during contraction. It is of interest

in this connexion that Hurley & Shelley (1954) mention the great difficulty of fixing material with the myoepithelium in the uncontracted state. By means of the intercellular spaces the contraction of the myoepithelium will circulate nutrient fluid obtained from the dermis, but as contractions occur only once in perhaps 24 hr. it is obvious that this cannot be the usual method of nourishing the secretory cells.

SUMMARY

A description is given of the electron microscopic histology of the apocrine sweat gland. The mechanism of apocrine breakdown, and the difficulties of ascertaining the nature of the secretion, are described. It is considered that the old view that there is eccrine and apocrine secretion by the gland is correct. The papillate nature of the cell surfaces lining the lumen of the duct, together with the relatively rich mitochondrial content of the cells and the innervation of the duct, suggests that the latter exerts some control on the composition or concentration of the sweat.

I am greatly indebted to Mr W. Crone, formerly of the Leeds General Infirmary, for his kindness in supplying the material.

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KEY TO ABBREVIATIONS

c., canaliculi; *c.d.*, cell debris; *c.r.*, clear region of cytoplasm; *d.*, dermis; *d.f.*, dark fibrils; *f.*, tonofibrils; *g.*, Golgi apparatus; *gr.*, secretory granule; *i.s.*, intercellular space; *lum.*, lumen; *mit.*, mitochondrion; *my.*, myoepithelium; *n.*, nucleus; *no.*, nucleolus; *p.*, papillae; *p.m.c.*, papillate apical membrane of secretory cell; *pr.*, prickle system; *r.*, reticulin; *r.g.*, rough secretory granule; *r.m.*, ruptured apical membrane; *s.g.*, smooth secretory granule; *s.m.c.*, non-papillate apical membrane of secretory cell; *s.o.*, smaller openings; *s.v.*, secretory vesicles; *v.*, vesiculate material.

EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Part of a transverse section of the secretory tubule. The Golgi apparatus is well seen in one cell. In the right-hand cells the apical membranes have ruptured and a clear region of so-called 'hyalin cytoplasm' has formed underneath. A transverse section through a clear region of the cell apex with ruptured membrane is shown (*t.s.*). $\times 4500$.
- Fig. 2. A slightly oblique transverse section at a level near the middle of the secretory cells. The canaliculi and papillae are well shown. $\times 10500$.

PLATE 2

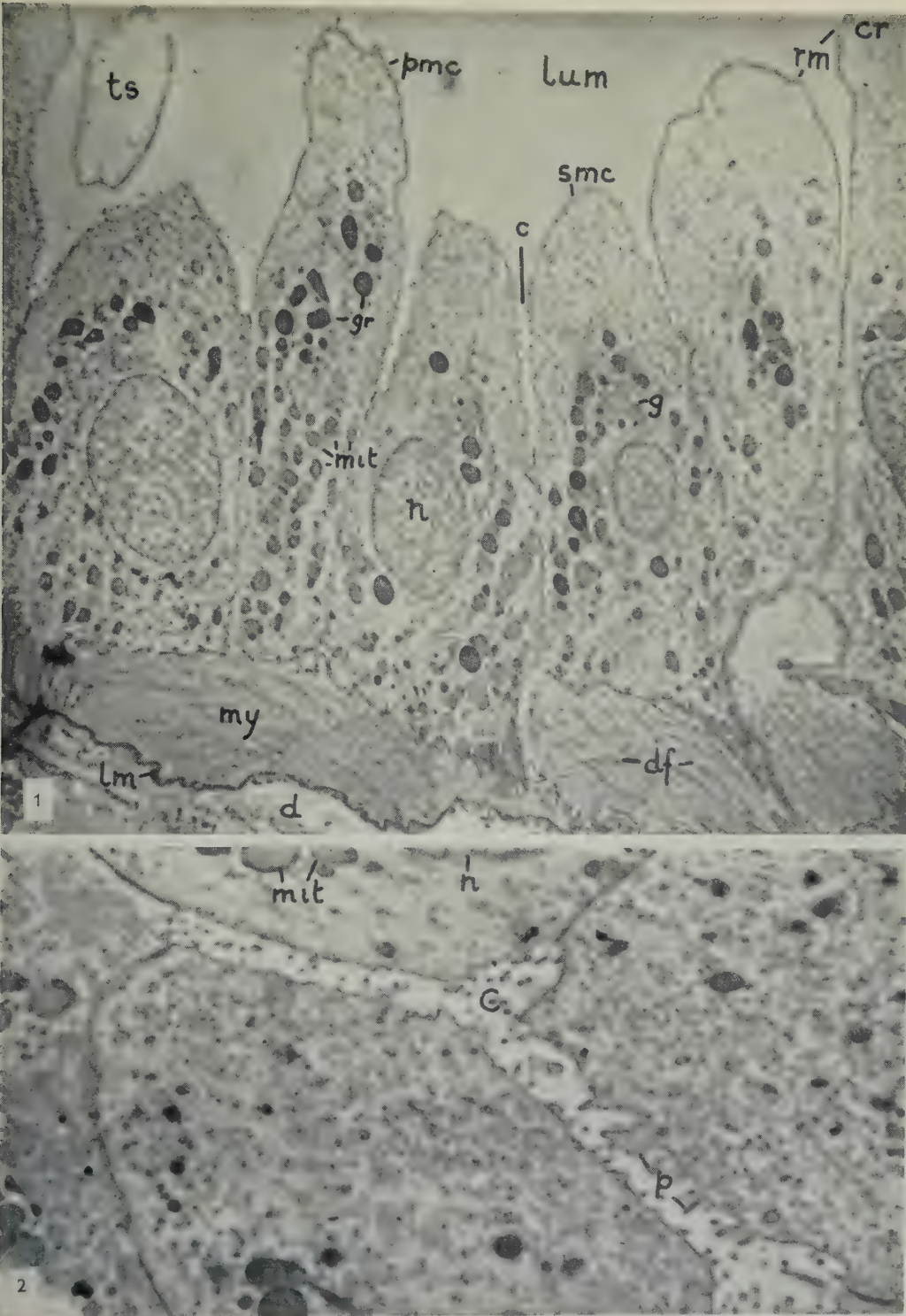
- Fig. 3. An enlargement to show the Golgi apparatus and secretory granules of a secretory cell. The particulate nature of the dense peripheral material of the rough granules is seen. $\times 24500$.
- Fig. 4. Showing the basal myoepithelial cells in transverse section. The dark fibrils appear as black dots. The moderately electron-dense cementing substance (*c.s.*) is almost absent at (*l.m.*), the limiting membrane between the gland and the dermis. A large intercellular space is shown between a myoepithelial cell and the base of the secretory cell. The basal membrane of the secretory cell is much folded, but its double nature cannot be seen because it is obliquely cut. $\times 14000$.
- Fig. 5. A longitudinal section of a secretory cell showing the complex infolding of its basal membrane, which forms the limiting membrane (*l.m.*). The cell is flanked by myoepithelial cells. The dense dots in this and succeeding figures are particles of gold sol about 160 A.U. diameter, used as an aid to focusing (Flewett & Tymms, 1956). $\times 33000$.

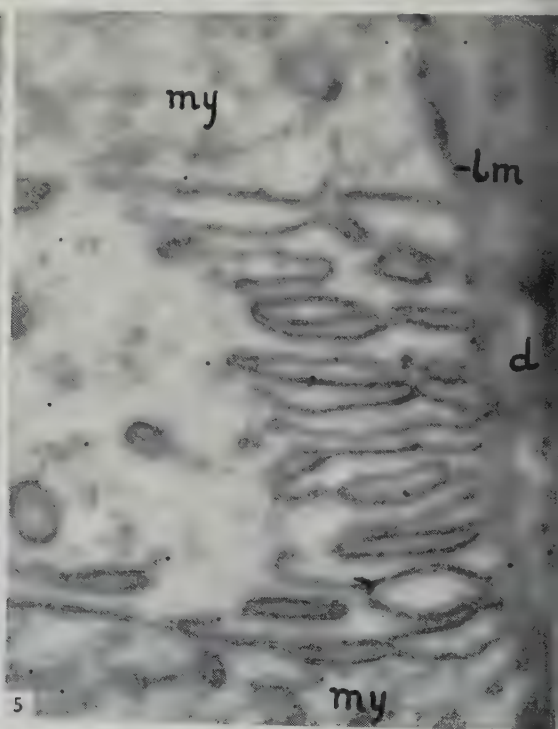
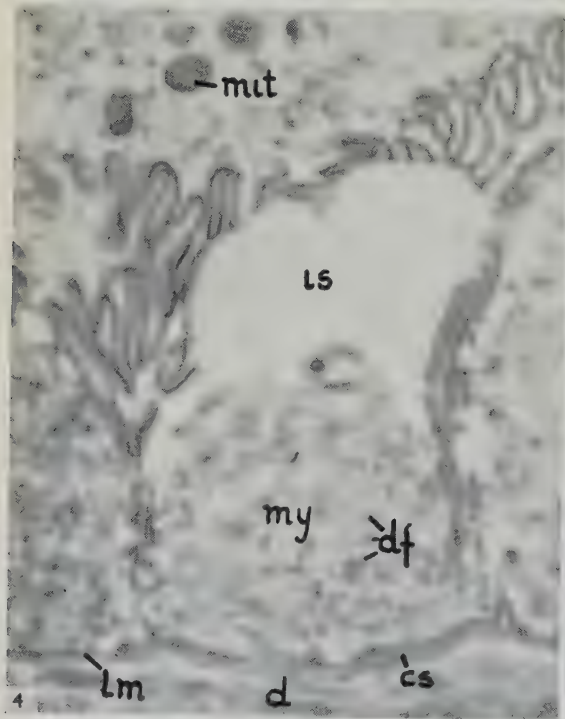
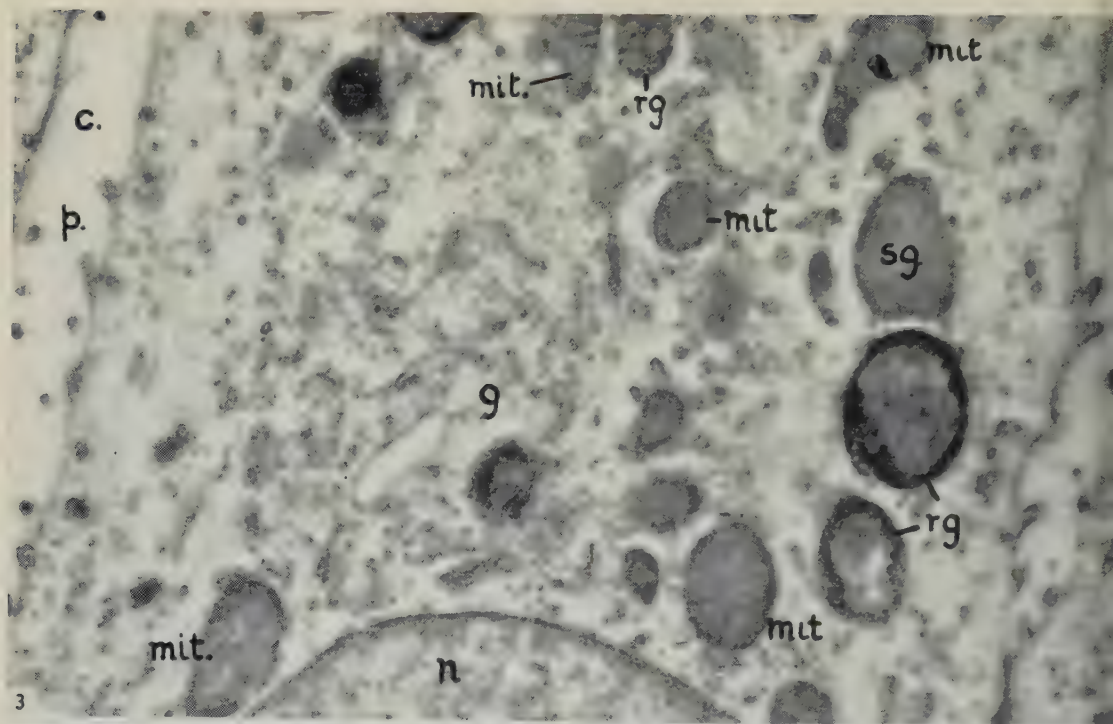
PLATE 3

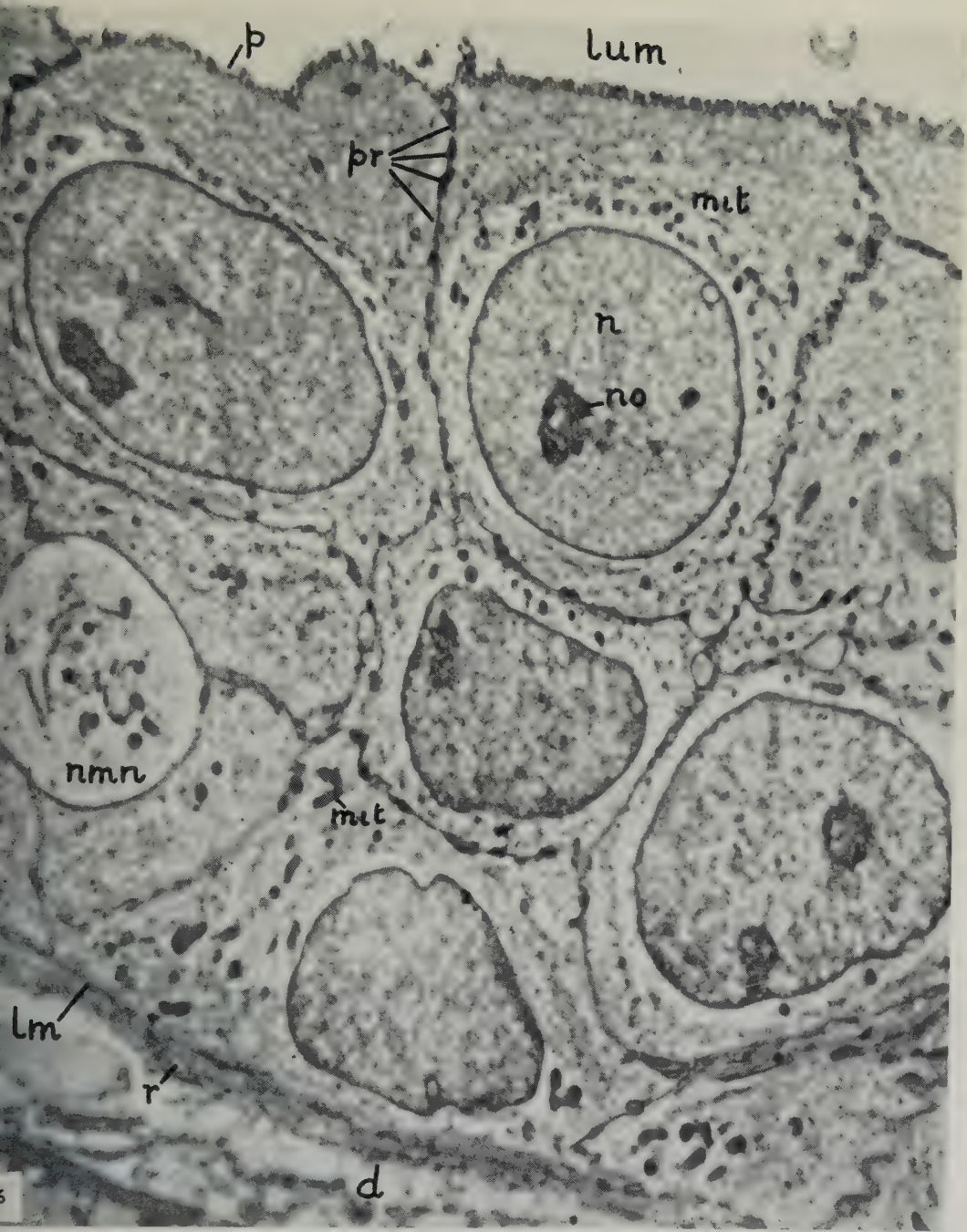
- Fig. 6. A section through the duct wall. The upper cells line the lumen and are papillate, while the lower cells are in contact with the dermis. An unmedullated nerve fibre is shown (*nmn.*). In one, possibly three, cells the dense material seen attached, or near, to the nuclear membrane is perhaps sex chromatin (Moore, Graham & Barr, 1953). $\times 10000$.

PLATE 4

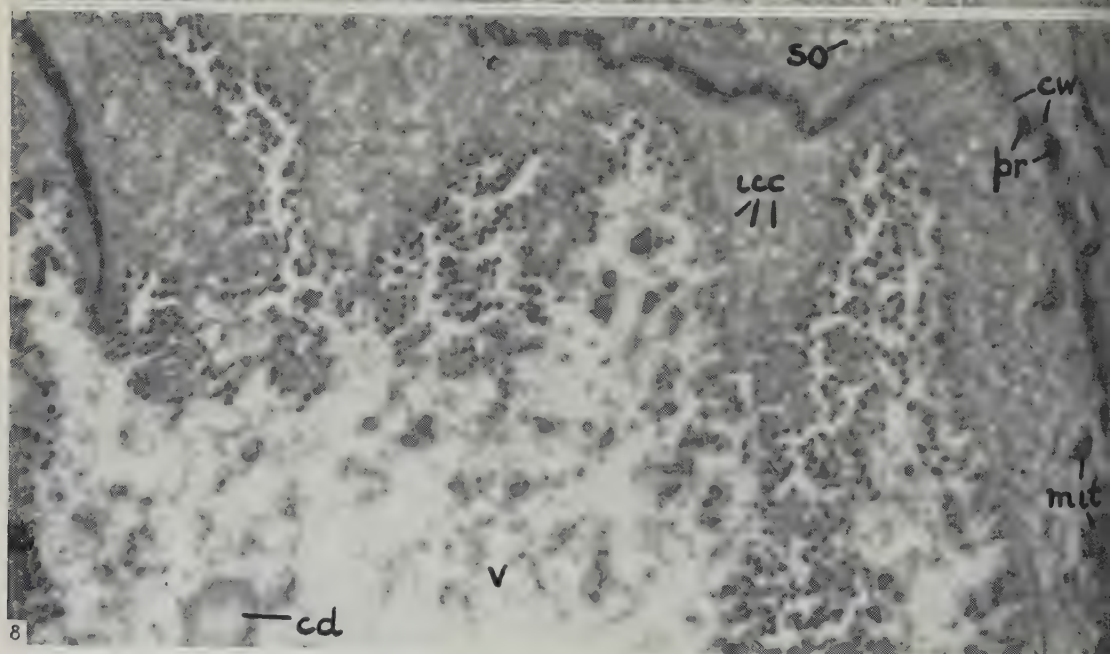
- Fig. 7. The opening of the apocrine duct in the hair follicle. The opening is very irregular and can only be partly shown in this figure, and there is some evidence of the secretory products of the gland. $\times 4500$.
- Fig. 8. Enlarged view of part of the region in the lower right corner of the previous figure. The vesiculate material is now more clearly seen, and is obviously different from the transversely sectioned papillae. Intracellular canals (*ic.c.*) give a sieve-like appearance to the cytoplasm of the cells immediately next to the lumen. Exceptionally numerous prickles with intervening cell walls (*c.w.*) are seen. $\times 15500$.







CHARLES—STUDY OF THE HUMAN AXILLARY APOCRINE GLAND



A STUDY OF THE MELANOCYTES AND MELANIN IN THE SKIN OF THE MALE GUINEA-PIG

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It is now agreed by most observers that melanin formation in all vertebrates takes place in specialized dendritic cells called melanocytes. The amount of melanin in the skin and hair is subject to considerable variation and is dependent on the numbers of melanocytes present and on the activity of these cells. Ever since Bloch's discovery of the Dopa reaction (1917) numerous investigations have been carried out in an attempt to explain the mechanisms involved in regulating the production of melanin. We are interested in studying the effect of the sex hormones on melanocytes and melanin production using carefully controlled histochemical experiments, and for this purpose we have chosen the guinea-pig as a suitable laboratory animal. Before investigating the effect of the male sex hormone on melanogenesis in the male guinea-pig, it was considered fundamental to the problem that a thorough knowledge of the numbers and morphology of the melanocytes and the amount of melanin present in the different skin regions in the normal animal at different stages of sexual development should first be obtained. During the course of this investigation we have also closely studied the precise relationship of the melanocytes and melanin granules to the other cells of the epidermis. To simplify the investigation we have confined our study to the melanocytes and melanin found in the surface epidermis, and have ignored completely the melanocytes and melanin of the hair follicles. The results of this preliminary work form the substance of this paper.

MATERIALS AND METHODS

Pure black and pure red male guinea-pigs were used and these were divided into three groups: (1) Twenty-four mature animals all weighing between 450 and 912 g. to study the melanocytes in the normal adult guinea-pig. (2) Seven immature animals all weighing under 265 g. and known to be less than 25 days old to study the melanocytes before the onset of sexual activity. (3) Seven animals weighing between 350 and 390 g. to study the melanocytes during the period of rapid sexual development (Deanesly & Rowlands, 1936).

Skin samples measuring about 0.5 cm.² were removed from all the animals from the ear, anterior abdominal wall and sole of foot; in addition the right areola was excised. Most of the skin samples were left intact after excess subcutaneous fat and the deeper part of the dermis had been excised under a dissecting microscope. A few of the samples were treated with trypsin to disengage the epidermis using the 'skin-splitting' technique of Billingham & Medawar (1951), as modified by Szabó (1955). All the samples were then processed under identical conditions. The skin was fixed in 5 % formol saline for 16 hr. and after washing it in normal saline for $\frac{3}{4}$ hr. it was incubated at 37° C. in a 1 in 1000 solution of L-dihydroxyphenylalanine at a pH

of 7.4. After 2 hr. the substrate was renewed and incubation continued for a further $1\frac{3}{4}$ hr. Fixation for a further period of 16 hr. in 10 % formol saline was then carried out, followed by dehydration and clearing. The greater part of each skin sheet was mounted in Canada Balsam, the remainder was embedded in paraffin and vertical sections $6\ \mu$ thick were cut. In order to identify the various layers of the epidermis a number of the vertical sections were counterstained with haematoxylin and eosin.

All the skin sheets were examined with the dermal surface uppermost except those of the anterior abdominal wall where, owing to the large number of hair roots present in the dermis, it was found more satisfactory to examine the sheets with the epidermis uppermost. The appearances of the melanocytes in the skin samples removed from the different groups of animals were studied by assessing the number and size of the melanocytes and the amount, colour and position of the melanin within the cells. The length, width and complexity of the dendritic processes were also noted and an attempt was made to assess the amount and colour of the free melanin present, i.e. that melanin which is situated outside the melanocytes. The skin sheets of the sole of the foot were examined only in the red animals, since the very high concentration of melanin present in the skin of the black animals in this region prevented their accurate assessment.

The melanocytes were counted by projecting the image of a skin area measuring 0.04 mm.^2 on to a ground-glass screen marked out in the form of a grid. Ten randomly chosen areas were counted at a magnification of 375. Black skin was less easy to count than red, because it possessed more and darker free melanin granules which tended to obscure the melanocytes. In the sole of the foot melanocyte counts were found to be impracticable owing to the melanocytes being superimposed one upon the other on the sides of the very steep dermal papillae.

RESULTS

The melanocytes of guinea-pig skin occur in two sites, the hair follicles and the basal layer of the surface epidermis. In the present work our study has been confined to the melanocytes and melanin of the surface epidermis.

MELANOCYTES AND MELANIN IN THE MATURE GUINEA-PIG

Skin of ear

Skin sheets. The melanocytes were found to be concentrated in the broad epidermal ridges and only a few were found over the narrow dermal papillae (Pl. 1, fig. 1). The melanocyte counts are set out in Table 1. The mean number of melanocytes per mm.^2 in the black animals ranged between 793 and 1340 (overall mean 1141 ± 47 s.e.) and in the red animals ranged between 793 and 1543 (overall mean 1067 ± 75 s.e.). The melanocytes (Pl. 1, fig. 2) were seen as ovoid cells possessing relatively short dendritic processes which extended laterally and upwards. The cell bodies showed considerable differences in size having a maximum diameter which varied between $8\text{--}18\ \mu$ and a minimum diameter which varied between $4\text{--}10\ \mu$. At the point where each dendrite emerged from the cell body it was seen to be thick, gradually becoming slender towards its end. Each dendrite usually divided mainly by dichotomy into primary, secondary and more branches. The number of parent

dendrites arising from the cell body varied from three to six, four dendrites being the commonest arrangement. The width of the dendrites at their point of origin from the cell body varied but some measured as much as $3\ \mu$. The total length of the dendrites was difficult to measure, but it was noticed that considerable variation occurred, the maximum length being as much as $48\ \mu$. Fusion between the terminal twigs of the dendritic processes originating from the same cell was not observed, but the terminal twigs of the dendritic processes originating from different melanocytes often appeared to fuse (see anterior abdominal wall melanocytes, Pl. 2, fig. 12). Many of the dendritic processes showed fusiform swellings along their course and some showed terminal swellings (Pl. 1, fig. 2). The swellings were least conspicuous in those dendritic processes which contained a large amount of melanin. At the centre of the cell body a rounded area containing less melanin could usually be observed and in specimens examined with the phase contrast microscope, this was seen to be due to the presence of the nucleus. The bodies of the melanocytes and their dendritic processes contained a moderate number of melanin granules which appeared to be of constant size and were most heavily concentrated at the periphery of the cytoplasm. Free melanin granules were also seen to be lying between the melanocytes and are found in greatest concentration in the vicinity of these cells (Pl. 1, fig. 1). The colour of the melanin granules varied from light brown to black, being darker in the black than in the red animals.

Vertical sections (Pl. 2, fig. 9). The epidermis of the ear consists of about six layers of cells and there are well-formed dermal papillae. The melanocytes were found mainly in the epidermal ridges and were seen to lie between the ordinary epidermal cells of the basal layer. The melanocytes were seen in profile as ovoid or round bodies, but the detailed morphology of the melanocytes and their processes was observed to very much better advantage in the skin sheets. In the basal layer the melanin granules appear to be distributed diffusely throughout the ordinary epidermal cells, but many of these apparently free melanin granules were thought in fact to lie within the dendritic processes of the melanocytes, which entwined themselves around and between the neighbouring epidermal cells. In the deeper cells of the stratum spinosum the free melanin granules were seen to be concentrated around the periphery of the cytoplasm, but in the more superficial cells of this region the granules were found to be concentrated mainly in the superficial poles of the cells. In the cells of the stratum granulosum and stratum lucidum the melanin was frequently seen to lose its polar distribution and the granules were more evenly distributed throughout the cytoplasm. In the stratum corneum the melanin granules were found to lie in the flattened squamous cells and were arranged in rows parallel with the surface. The arrangement of the melanin granules in the epidermis is similar in all the regions, but is seen to best advantage in sections of the sole of the foot owing to the greater thickness of the epidermis (Pl. 2, fig. 16).

Skin of anterior abdominal wall

Skin sheets. The melanocytes in most of the animals were found to be concentrated in rows which ran at right angles to the slope of the hairs (Pl. 1, fig. 3). The melanocyte counts for each animal are set out in Table 1. The mean number of melanocytes per mm.² in the black animals ranged between 143 and 458 (overall

mean 305 ± 27 S.E.) and in the red animals ranged between 65 and 335 (overall mean 165 ± 25 S.E.). The appearances of the melanocytes (Pl. 1, fig. 4) were similar to those seen in the skin of the ear, but the cell bodies were smaller and the majority possessed fewer but longer dendritic processes. The width of the dendrites at their point of origin from the cell body was smaller than that of the melanocytes of the ear being 1 to 2μ across. The appearances of the melanin granules seen in the melanocytes were similar to those of the ear but the concentration of the granules was very much lower. The concentration of the free melanin granules was also found to be lower than that of the ear and was present in greatest amount in the immediate vicinity of the dendritic processes.

Table 1. *Melanocyte counts in the skin of mature male guinea-pigs*

No. of animal	Colour of animal	Weight of animal (g.)	Melanocytes per mm. ²		
			Ear	Anterior abdominal wall	Areola
141	Black	560	1323	323	920
142	Black	466	1210	293	753
143	Black	573	1198	208	905
144	Black	474	1250	300	750
300	Black	544	1135	458	1058
301	Black	632	908	448	963
303	Black	520	793	143	—
304	Black	456	1043	243	848
305	Black	487	1305	228	653
306	Black	462	1340	298	913
340	Black	679	1255	380	715
341	Black	522	1073	403	730
342	Black	634	1000	235	760
131	Red	450	1355	120	1010
132	Red	480	1040	165	588
311	Red	453	805	88	550
312	Red	497	1543	230	728
313	Red	639	793	113	680
314	Red	548	1155	263	815
316	Red	912	950	65	448
317	Red	584	905	335	518
350	Red	524	895	155	573
351	Red	566	950	105	465
352	Red	569	1345	178	675
Black animals					
Mean melanocyte count per mm. ²			1141	305	831
Standard error			47	27	35
Red animals					
Mean melanocyte count per mm. ²			1067	165	641
Standard error			75	25	50
Difference in mean cell counts per mm. ² between black and red animals			74 ($P > 0.4$)	140 ($P < 0.001$)	190 ($P < 0.01$)

Vertical sections (Pl. 2, fig. 11). The epidermis of the abdominal skin is thinner than that of the ear consisting of only two to four layers of cells in addition to the stratum corneum. The melanocytes were seen to be concentrated mainly in that region of the epidermis immediately adjacent to the hair follicles and were situated between the ordinary epidermal cells of the basal layer. The cell bodies of the melanocytes seen in profile were smaller than those of the ear. The free melanin

granules were few in number and were found mainly in the vicinity of the melanocytes; in the stratum corneum the granules become more diffusely arranged lying in rows parallel with the surface.

Skin of areola

Skin sheets. The melanocytes were found mainly in the epidermal ridges (Pl. 2, fig. 14) and were present in greatest numbers towards the nipple. The epidermal ridges were seen to radiate out from the nipple towards the periphery of the areola where they gradually become wider and less pronounced. The edges of the epidermal ridges were darker in colour and this appeared to be due to a high local concentration of melanocytes and melanin (Pl. 1, fig. 5). The melanocyte counts were carried out about halfway between the nipple and the periphery of the areola and are shown in Table 1. The mean number of melanocytes per mm.² in the black animals ranged between 653 and 1058 (overall mean 831 ± 35 S.E.) and in the red animals ranged between 448 and 1010 (overall mean 641 ± 50 S.E.). The melanocytes (Pl. 1, fig. 6) resembled those of the anterior abdominal wall, but the cell bodies were larger and were seen to possess a greater number of dendritic processes which were both wider and longer. The amount of melanin present in the melanocytes and the amount of free melanin was less than that found in the ear but greater than that in the anterior abdominal wall. It was interesting to note that the melanocytes situated near the nipple tended to have smaller cell bodies and shorter dendritic processes than those situated in the middle zone of the areola; towards the periphery the melanocytes more closely resembled those of the anterior abdominal wall.

Vertical sections (Pl. 2, fig. 13). The epidermis of the areola consists of two to five layers of cells in addition to the stratum corneum. The melanocytes were found between the ordinary epidermal cells of the basal layer. The epidermal ridges and dermal papillae are well formed in this region being most marked near the nipple. The melanocytes were observed to be practically confined to the epidermal ridges and were found in greater concentration towards the nipple. The apparent high local concentration of melanocytes and melanin seen at the dark edges of the epidermal ridges in the skin sheets, was now seen to be due to the fact that when viewed from the dermal surface, the melanocytes and melanin granules are superimposed on one another as they lie on the sides of the epidermal ridges. The light areas which were present between the epidermal ridges were due to the melanocytes and melanin being practically absent over the dermal papillae. The bodies of the melanocytes seen in profile had a similar appearance to those of the ear. The free melanin granules had a similar distribution to that found in other regions, but there were more present than in the anterior abdominal wall and less than in the ear.

Skin of sole of foot

Skin sheets. The melanocytes were found to be concentrated mainly on the epidermal ridges (Pl. 1, fig. 7). The appearances of the melanocytes (Pl. 1, fig. 8) were similar to those seen in the skin of the ear, but the cell bodies tended to be smaller and the dendritic processes were more angular and branched more profusely. The amount and arrangement of the melanin granules in the bodies of the melanocytes and their dendritic processes were seen to be similar to that of the ear but the

colour of the melanin appeared lighter. The concentration of the free melanin also closely resembled that of the ear.

Vertical sections (Pl. 2, fig. 15). The epidermis of the sole of the foot consists of thirteen to twenty-three layers of cells in addition to a very thick stratum corneum, and very well-marked epidermal ridges and dermal papillae are present. The melanocytes were seen to be concentrated in the epidermal ridges and only a few were found over the dermal papillae. They were seen to lie between the ordinary epidermal cells of the basal layer. The bodies of the melanocytes were rounded in shape. In all the layers of the epidermis the free melanin granules had a similar appearance and arrangement to that found in the ear (Pl. 2, fig. 16).

Although it was impossible to carry out accurate melanocyte counts on the skin of the sole of the foot, for the reasons stated previously, it was clear that there were far more present in the black than in the red animals. A comparison of the skin sheets and vertical section with those of other regions examined indicated that there were more present in the sole than elsewhere.

MELANOCYTES AND MELANIN IN THE IMMATURE GUINEA-PIG

In the skin of the ear, the melanocytes were concentrated mainly in the epidermal ridges, as in the mature animal, but since the dermal papillae are less pronounced the distribution appeared to be more uniform. The cell counts are shown in Table 2 and it is seen that they showed a tendency to be higher than in the mature animal. The cell bodies of the melanocytes in most of the immature animals contained more melanin and the dendritic processes were shorter than those of the mature animals.

Table 2. *Melanocyte counts in the skin of immature male guinea-pigs*

No. of animal	Colour of animal	Weight of animal (g.)	Melanocytes per mm. ²		
			Ear	Anterior abdominal wall	Areola
2	Black	264	1300	398	1008
3	Black	165	1283	490	988
4	Black	150	1500	385	763
5	Black	259	1373	413	940
11	Red	257	1135	213	665
12	Red	220	985	123	675
13	Red	140	1420	110	443
Black animals: mean cell count per mm. ²			1364	422	925
Standard error			49	24	56
Red animals: mean cell count per mm. ²			1180	149	594
Standard error			128	32	76

In the anterior abdominal wall the melanocytes showed less tendency to be concentrated in the region of the hair follicles and, as a result, had a more general distribution than those of the mature animal (Pl. 2, fig. 10). The cell counts are shown in Table 2. The melanocytes appeared to contain more melanin than those found in the adult, and the size of the cell bodies and the length and complexity of the dendritic processes were slightly greater (Pl. 2, fig. 12). In the areola the general distribution of the melanocytes was found to be very similar to that of the adult animals, but the cell bodies of the melanocytes appeared to contain more melanin.

The cell counts of the skin of the areola are shown in Table 2. In the sole of the foot the general appearances and distribution of the melanocytes were practically identical to those found in the adult animals.

In all the skin regions examined in the immature guinea-pigs the amount, colour and distribution of the free melanin were seen to be very similar to those found in the mature male animals.

In the group of guinea-pigs which were studied during the period of rapid sexual development the appearances of the melanocytes and melanin in all the regions resembled those of the mature animal but, as would be expected, where differences between the mature and immature animals existed it was found that the appearances in this group lay somewhere between the two. The melanocyte counts of all the regions are shown in Table 3.

Table 3. *Melanocyte counts in the skin of the male guinea-pig during the period of rapid sexual development*

No. of animal	Colour of animal	Weight of animal (g.)	Melanocytes per mm. ²		
			Ear	Anterior abdominal wall	Areola
120	Black	370	990	333	628
121	Black	390	1090	493	765
122	Black	387	1123	425	1038
123	Black	363	1378	418	968
111	Red	377	1088	173	—
112	Red	350	1273	133	625
113	Red	350	923	10	510
Black animals: mean cell count per mm. ²			1145	417	850
Standard error			83	33	94
Red animals: mean cell count per mm. ²			1095	105	568
Standard error			101	49	58

DISCUSSION

In the adult male guinea-pig the melanocytes in the epidermis of the ear, areola and sole of foot were found to be practically confined to the epidermal ridges. In the anterior abdominal wall, where the epidermal ridges and dermal papillae are not well formed, the melanocytes were found to be concentrated in the epidermis immediately adjacent to the hair follicles. These observations are in agreement with those reported by Billingham (1948) and Billingham & Medawar (1953) for the ear and anterior abdominal wall of the guinea-pig. They were of the opinion, however, that the melanocytes of the anterior abdominal wall skin were confined to that part of the epidermis which abuts against the obtuse angle of the emergent hair. Although this arrangement has been observed in some sections in the present work it has not been a constant finding, for the melanocytes were frequently found to be equally distributed on both sides of the hair and on occasions were even found to be more heavily concentrated on the side which formed the acute angle with the emergent hair. In the areola well-formed epidermal ridges were seen to radiate from the nipple and fade out towards the periphery of the areola. The concentration of the melanocytes and melanin in these ridges gave them the appearance of radiating dark bands. In the immature animals the melanocytes had a more general distri-

bution in all the regions, and this was seen to be due to the fact that the epidermal ridges and dermal papillae were less well developed in these animals.

The melanocyte counts showed that there were wide individual and regional variations. The counts for the ear for example in different black adult animals varied from 793–1340 mm.². The melanocyte counts for the different regions in each animal showed that the population density is greatest in the skin of the ear, less in the areola and least in the anterior abdominal wall. Although it was impossible to carry out accurate melanocyte counts on the skin of the sole of the foot for the reasons stated previously, a comparison of the skin sheets and vertical sections with those of the other regions examined indicated that there were more present in the sole of foot than elsewhere. This could be explained by the presence of the large number and depth of the epidermal ridges in the sole of the foot, which results in the surface area of the basal layer of the epidermis being very much more extensive per unit area of skin surface than found in the other parts of the body, and consequently, many more melanocytes would be present in a given area of skin in this region. The counts for the ear and anterior abdominal wall are generally in agreement with those reported by Billingham & Medawar (1953) for guinea-pigs with red and black patches, although some of their counts for the anterior abdominal wall were higher.

In the present investigation it has been found that the number of melanocytes in the anterior abdominal wall and areola is significantly higher in the black than in the red animals; a similar tendency was noted in the skin of the ear and sole of foot. In addition, in all the regions the black animals possessed more and darker melanin. Billingham & Medawar (1953) estimated the number of melanocytes in guinea-pig ear skin and general body skin and found that differences in colour are not associated with differences in density of melanocytes. These observations were carried out on patched red and black or red, white and black guinea-pigs and the comparisons were always made on skins of different colours removed from the same animal. In the present work the counts were always made on skin removed from the same area of a given region in different animals (either pure black or pure red) and this may account for the different results obtained. It is possible that the difference between melanocyte counts in the black and the red guinea-pigs might be due to genetical differences between the two strains not necessarily connected with pigmentation as such.

In human skin, Billingham (1949) and Staricco & Pinkus (1957) using skin sheets have concluded from the limited material at their disposal that the melanocytes of negro skin are no more abundant than those of white skin. However, the melanocyte counts carried out by Szabó (1954), Staricco & Pinkus (1957) and Breathnach (1957) have made it clear that in the human subject there are also wide individual and regional variations in melanocyte concentrations. In addition, as Breathnach points out, there may be considerable variations in the counts within a given region in the same individual. Thus, for differences in the melanocyte counts in a given skin region from different individuals to become apparent only those counts taken from the same area within the region should be compared. We feel that only then will it be possible to state positively that normal human skin of all colours contains the same number of melanocytes.

It was interesting to note that in both the black and red guinea-pigs the mean melanocyte count of the ear skin was higher in the immature than in the mature animals and the difference between the two groups was greatest in the black animals. In the anterior abdominal wall and areola of the black guinea-pigs the count was also higher in the immature than in the mature animals. Since the number of immature animals representing each colour was small, this finding must be treated with caution.

The general morphology of the melanocytes and the appearances of the melanin in the skin of the ear and anterior abdominal wall in the present work are generally in agreement with the findings of Billingham (1948) and Shukla, Karkun & Mukerji (1954). Any minor differences which existed may be attributed to the fact that both Billingham & Shukla and his co-workers were examining a suspension of free melanocytes, which in Billingham's investigation were living cells, whereas our studies have been confined to skin which had been formalin fixed with the melanocytes *in situ*.

In the skin of the areola the melanocytes were seen to be more numerous and larger and more melanin was present than in the general skin of the anterior abdominal wall, and these factors together almost certainly account for the colour of the areola being darker than that of the surrounding skin in the majority of the animals. It was interesting to find that the melanocytes in the areola were smaller towards the nipple, larger in the middle zone of the areola and at the periphery they were again small and assumed many of the characteristics of the anterior abdominal wall melanocytes. In the sole of the foot the melanocytes resembled those found in the ear skin, but the dendritic processes were more angular and branched more profusely.

Age appears to have relatively little effect on the general appearances of the melanocytes in the different skin regions of the guinea-pig. In the immature animals the amount of melanin present within the melanocytes was greater than in the mature animals, and this difference was most obvious in the black animals. This would appear to indicate that in the immature animals the melanocytes are capable of greater melanogenic activity than in the mature animals. In this connexion, it is of interest to note that it is the black immature animals which have the largest number of melanocytes in all the regions.

In each skin region the majority of the melanocytes presented certain characteristic features which were constant for that region and were found in all the animals examined. For example, in the skin of the ear the melanocytes tended to be star-shaped with large cell bodies and short stumpy processes, whereas in the anterior abdominal wall the cell bodies were smaller and possessed longer tenuous processes. However, a considerable variation in the size of the melanocytes within a given skin region was noted. A marked difference in the size of the cells within a particular region was also found in human skin by Szabó (1954) and Staricco & Pinkus (1957). The bodies of the melanocytes in all the skin regions were found to lie in the basal layer of the epidermis between the other epidermal cells of this layer. While the majority of workers in this field accept this fact in both human and animal skin, a few are still of the opinion that they lie a little deeper at the dermo-epidermal junction (Shukla, Karkun & Mukerji, 1954) or are just deep to the basal layer of the

epidermis (Becker, 1953). This difference of opinion may be due to the fact that not all observers have used the same techniques. For example, it is not difficult to imagine that in skin specimens where the epidermis is disengaged from the dermis by trypsin digestion some of the melanocytes are pulled upon by the removal of the dermis. In the present work the dendrites of the melanocytes were seen to extend out laterally in all directions parallel to the dermal surface or upwards between the other cells of the basal layer and the deeper cells of the stratum spinosum. It was seen that the cells gave off more dendrites in a lateral than in a perpendicular direction. In the ear and areola where the melanocytes are relatively close to one another it was noted that many of them appear to be joined together by their dendrites to form a syncytium. In the anterior abdominal wall, where the cells are found to be farther apart, only an occasional union between melanocytes was seen.

The button- or club-like expansions found on the ends of the terminal branches of the dendrites described by Masson (1948) and Billingham (1948) and their application to the walls of other basal cells was also observed in the present work; they were not seen, however, on all the dendritic processes. The fusiform swellings noted along the lengths of the dendritic processes were believed to be due to local aggregations of melanin granules. Staricco & Pinkus (1957) also observed these swellings in the dendrites of human melanocytes and they tentatively suggested that they might represent successive crops of melanin granules migrating along the dendrites from the cell body. Observation of living melanocytes in tissue culture would probably provide proof of this movement of melanin.

The melanin granules found in association with the ordinary epidermal cells of the basal layer have been the subject of much speculation. Masson (1948), Stearner (1946) and Billingham (1948) hold the view that the pigment granules have been inoculated into the neighbouring epidermal cells through the terminal caps of the dendritic processes. This melanin secreting activity was called 'cytocrine' by Masson. Becker, Fitzpatrick & Montgomery (1952), on the other hand, have challenged this theory. They believe that the melanin in the epithelial cells is illusory and that the pigment is really in the dendritic processes which are wrapped around each basal cell. Observations in the present work lead us to believe that while some of the melanin is actually in the cytoplasm of the basal epidermal cells the remainder, however, appears to be in the dendritic processes of the melanocytes. This may account for the fact that the melanin content of the more superficial cells appears to be lower than that of the basal layer cells, since as the cells pass upwards through the epidermis, they gradually lose contact with the melanin-filled dendrites of the melanocytes.

SUMMARY

1. The melanocytes and melanin in the skin of the ear, anterior abdominal wall, areola and sole of foot have been studied in pure black and pure red male guinea-pigs. The melanocytes were identified by using the Dopa reaction. The melanocytes and melanin present in the hair follicles have not been examined in this investigation.

2. In the ear, areola and sole of foot the melanocytes were found to be concentrated in the epidermal ridges. In the anterior abdominal wall the melanocytes were

situated in rows lying at right angles to the slope of the hairs and were mainly found in the epidermis lying adjacent to the opening of the hair follicles.

3. The melanocyte counts showed that there were wide individual and regional variations. The cell counts were greatest in the skin of the ear, less in the areola and least in the anterior abdominal wall. In the sole of the foot accurate counting was not possible, but more melanocytes appeared to be present in this region than elsewhere.

4. In the black animals the number of melanocytes in the anterior abdominal wall and areola was significantly greater than in the red animals; a similar tendency was noted in the skin of the ear and sole of foot. The black animals were also seen to possess a greater amount of melanin in all the regions.

5. The melanocytes were found to lie between the cells of the basal layer of the epidermis, and the dendritic processes were seen to extend out laterally and upwards between the deeper cells of the epidermis.

6. The majority of the melanocytes present in each skin region presented certain characteristics which were constant for that region and were found in all the animals examined.

7. In the immature animals the melanocytes had a more general distribution than those found in the mature animals; this was seen to be due to the fact that the epidermal ridges and dermal papillae were less developed in the immature animals. In the black immature animals the mean melanocyte counts for the skin of the ear, anterior abdominal wall and areola were higher than in the black mature animals, but in the red immature animals the melanocyte count was only higher in the skin of the ear. The amount of melanin present within the melanocytes was greater in the immature guinea-pigs and this was most obvious in the black animals.

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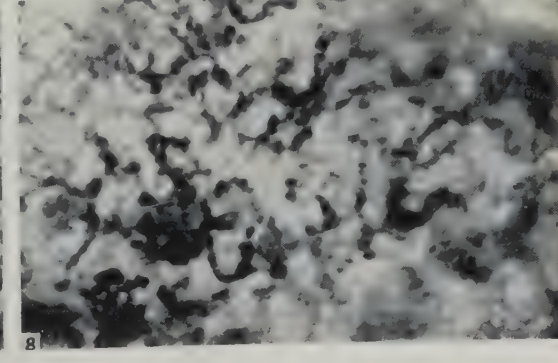
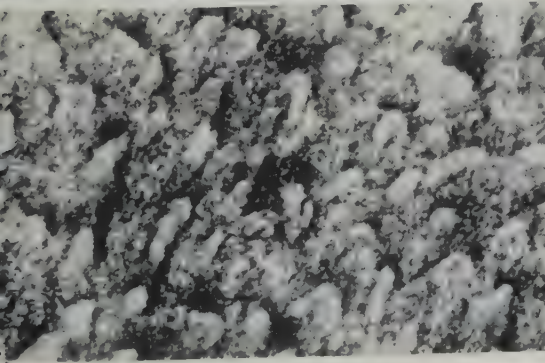
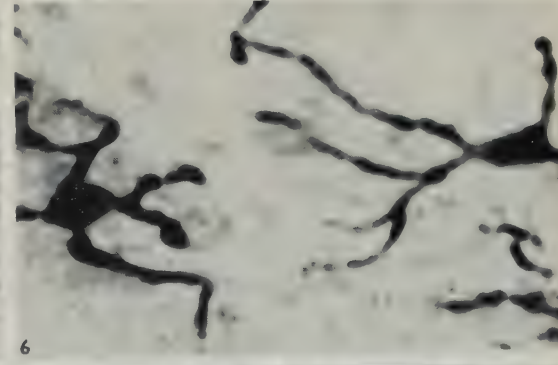
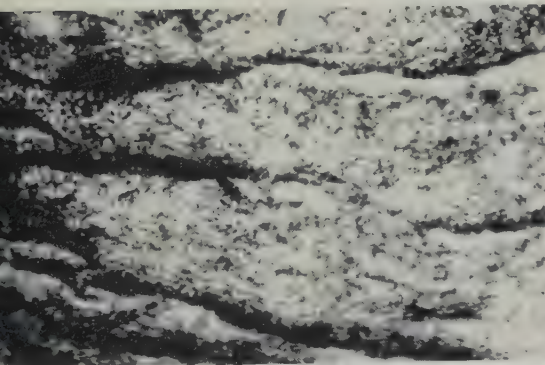
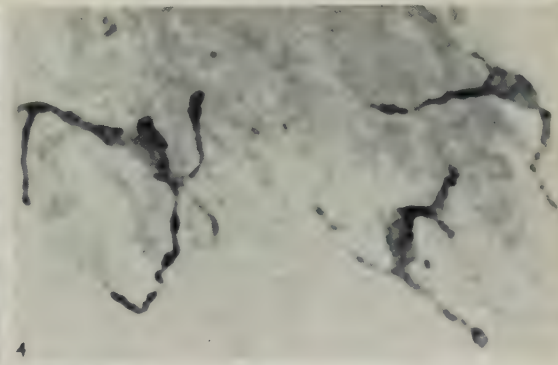
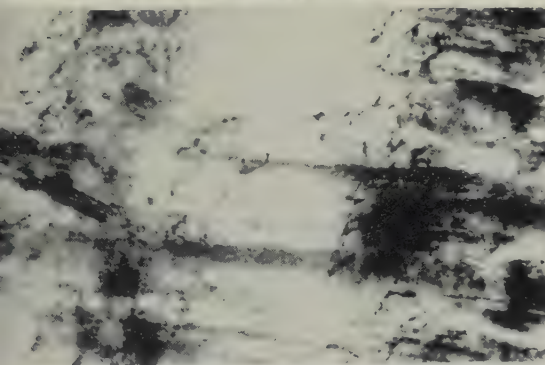
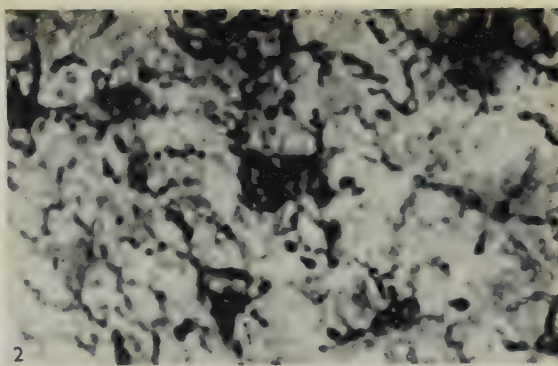
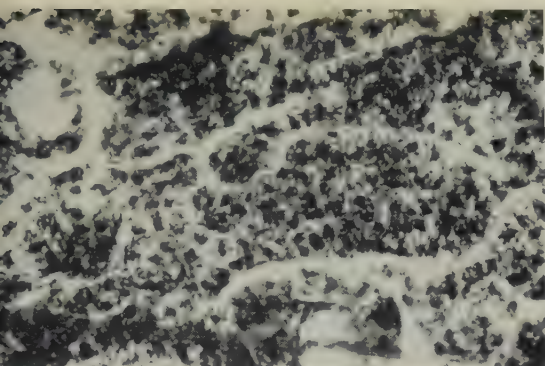
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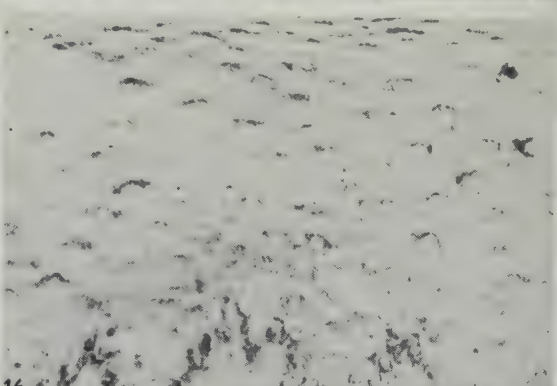
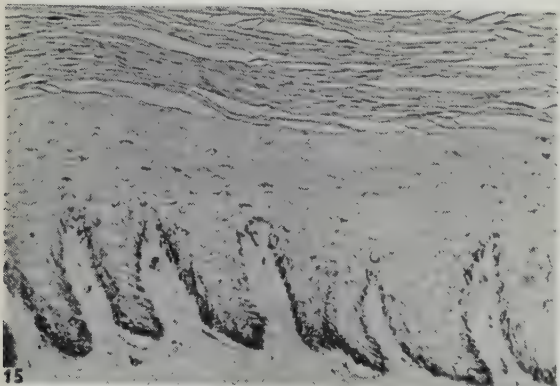
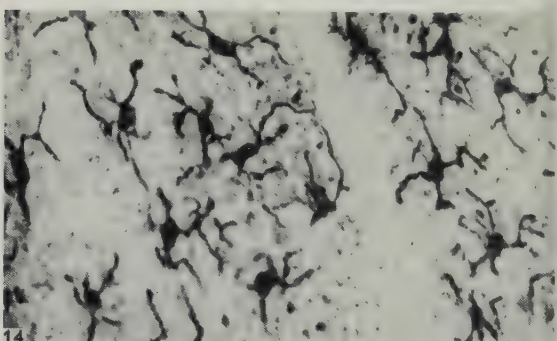
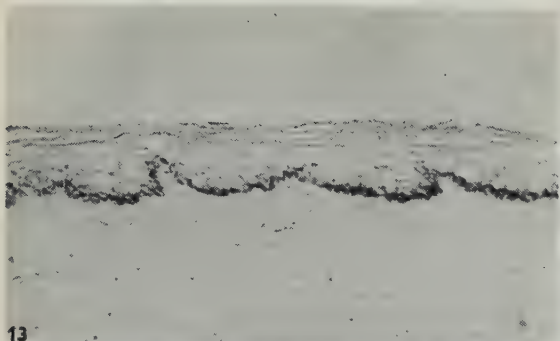
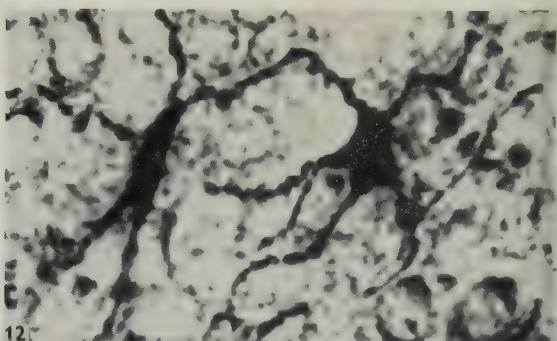
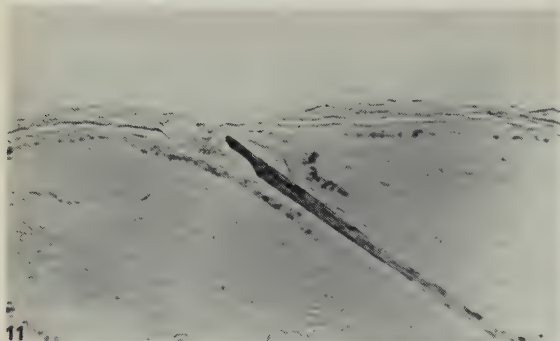
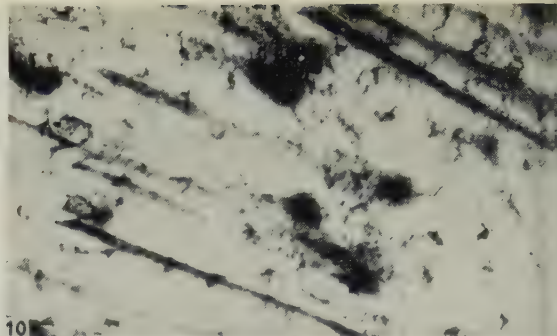
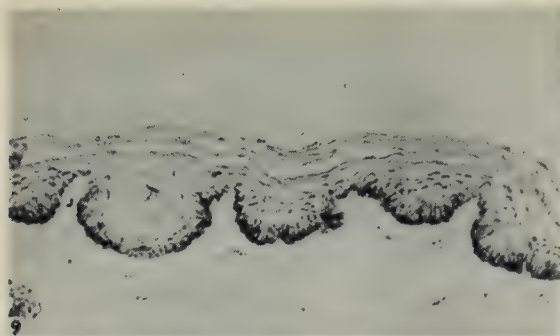
PLATE 1

- Fig. 1. Photomicrograph of skin sheet of ear of black mature male guinea-pig. Shows melanocytes and melanin to be practically confined to the broad epidermal ridges; the dermal papillae appear as white bands owing to the relative absence of melanocytes and melanin in these areas. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 120$).
- Fig. 2. High-power photomicrograph of skin sheet of ear of red mature male guinea-pig. Shows star-shaped melanocytes with large, ovoid cell bodies and short stumpy processes. Note also the presence of a number of melanocytes with small cell bodies. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 800$).
- Fig. 3. Photomicrograph of skin sheet of anterior abdominal wall of black mature male guinea-pig. Shows melanocytes and melanin to be concentrated in rows which run at right angles to the slope of the hairs. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 120$).
- Fig. 4. High-power photomicrograph of skin sheet of anterior abdominal wall of red mature male guinea-pig. Shows melanocytes with small cell bodies and possessing few but long dendritic processes. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 800$).
- Fig. 5. Photomicrograph of skin sheet of areola of red mature male guinea-pig. Shows melanocytes and melanin to be concentrated mainly on the epidermal ridges which radiate from the nipple towards the periphery of the areola. The epidermal ridges are seen to have dark edges. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 120$).
- Fig. 6. High-power photomicrograph of skin sheet of areola of red mature male guinea-pig. Shows melanocytes with large cell bodies and very long, wide dendritic processes. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 800$).
- Fig. 7. Photomicrograph of skin sheet of sole of foot of red mature male guinea-pig. Shows melanocytes and melanin to be concentrated mainly on the epidermal ridges. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 120$).
- Fig. 8. High-power photomicrograph of skin sheet of sole of foot of red mature male guinea-pig. Shows melanocytes with large cell bodies and possessing many angular dendritic processes. Owing to the steepness and extreme height of the dermal papillae many of the melanocytes are out of focus. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 800$).

PLATE 2

- Fig. 9. Photomicrograph of vertical section of skin of ear of black mature male guinea-pig. Shows melanocytes to be situated mainly in the epidermal ridges and to lie in the basal layer of the epidermis. Note that the melanin is concentrated in the superficial poles of the cells in the upper layers of the stratum spinosum. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 146$).
- Fig. 10. Photomicrograph of skin sheet of anterior abdominal wall of black immature male guinea-pig. Shows diffuse distribution of melanocytes. Compare with anterior abdominal wall of mature animal (see Fig. 3). Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 116$).
- Fig. 11. Photomicrograph of vertical section of skin of anterior abdominal wall of black mature male guinea-pig. Shows melanocytes to be distributed approximately equally on both sides of the emergent hair and to lie in the basal layer of the epidermis. Note the relative absence of melanin in this region. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 146$).





- Fig. 12. High-power photomicrograph of skin sheet of anterior abdominal wall of black immature male guinea-pig. Shows two melanocytes and many free melanin granules lying outside these cells. Note the larger size of the cell bodies and the greater length and complexity of the dendritic processes as compared with the melanocytes of the anterior abdominal wall of the mature animal (see Fig. 4). Note also that two of the dendritic processes appear to be in continuity. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 800$).
- Fig. 13. Photomicrograph of vertical section of skin of areola of black mature male guinea-pig. Shows melanocytes to be concentrated mainly in the epidermal ridges and to lie in the basal layer of the epidermis. Note that there is more melanin present than in the anterior abdominal wall (Fig. 11) and less than in the ear (Fig. 9). Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 146$).
- Fig. 14. Photomicrograph of skin sheet of areola of red mature male guinea-pig. Shows the melanocytes to be practically confined to the epidermal ridges and that the melanin granules are most heavily concentrated in their vicinity. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 329$).
- Fig. 15. Photomicrograph of vertical section of skin of sole of foot of black mature male guinea-pig. Shows the melanocytes to be situated mainly in the epidermal ridges and to lie in the basal layer of the epidermis. Note that the melanin is concentrated in the superficial poles of the cells in the upper layers of the stratum spinosum. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 146$).
- Fig. 16. High-power photomicrograph of vertical section of skin of sole of foot of black mature male guinea-pig. Shows the arrangement of melanin granules in the various cell layers of the epidermis. Full thickness skin preparation treated with Dopa reagent. No counterstain ($\times 488$).

THE NASAL GLANDS OF BIRDS: A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE INACTIVE GLAND IN THE DOMESTIC DUCK

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INTRODUCTION

The nasal glands of birds are bilaterally paired structures whose ducts open into the nasal cavity. They are found near to or within the orbit, and since, in many species, they lie on the frontal bones, above the orbit, they are alternatively called the supraorbital glands. In most species each gland is itself double, although the two moieties are frequently fused within a common capsule of connective tissue. The dual nature of the gland is revealed, in section, by the presence of two main ducts; the medial duct opens on the surface of the nasal septum, the lateral into the vestibule. In some species the glands are very much reduced or even absent, in others only one of the two moieties is present on each side.

The gross morphology of the gland was reviewed in detail by Technau (1936), whose work clearly established the important general principle that the glands are larger in aquatic than in terrestrial birds, and are largest in marine species. The microscopic anatomy of the gland was described by Marples (1932) in a number of species. He confirmed Technau's (1936) finding of the maximal development of the gland in sea birds, and showed that the gland is a compound tubular one.

Technau's (1936) comparative studies seemed to provide strong support for the generally held view that the function of the gland is to irrigate the nasal mucosa; the large glands of marine birds were thought to protect the mucosa from the (presumably) irritant effects of sea water. Marples (1932) also subscribed to this view, although he claimed that the secretion was 'slimy' rather than watery. In all the species which he examined he found cellular debris within the ducts, and he thought that the secretion consisted of degenerating cells and that the gland was, therefore, holocrine in type.

The present study was undertaken because, in an investigation of the nasal cavity of the gannet (Scothorne, 1958), I was impressed by the large size and unusual structure of the gland, which appeared to be neither mucous nor serous in type, as far as could be judged from routinely stained preparations. It was decided, therefore, to study the gland histochemically and cytologically, using the duck in preference to the rather inaccessible gannet. This work was in progress, and it already had become evident that the gland was of a most unusual histological type, when my attention was drawn to the physiological studies of Schmidt-Nielsen and his co-workers (Schmidt-Nielsen, Jørgensen & Osaki, 1958; Schmidt-Nielsen & Sladen, 1958). In the cormorant and penguin they showed that an excessive oral or parenteral intake of sodium chloride provoked the secretion into the nasal cavity of a practically pure and strongly hypertonic solution of sodium chloride. They were

justifiably cautious about the precise origin of the secretion, but thought it 'likely that the nasal gland plays the major role' (Schmidt-Nielsen *et al.* 1958).

In the light of these physiological studies, the histological findings of the present study assumed a fuller significance; as will emerge in the discussion, the structure of the nasal gland is entirely compatible with the functional activity provisionally ascribed to it by Schmidt-Nielsen and his co-workers. Moreover, it will be shown that in the duck, also, salt-loading provokes the secretion of a hypertonic sodium chloride solution into the nasal cavity.

MATERIAL AND METHODS

The histological studies were made on glands from five ducks, two of which were immature birds (aged about 4 months) whose sex was not noted, the other three adult females. The functional studies were made on two adult females. All birds were of the Aylesbury strain.

The following methods were employed:

I. General histology

Fixation in either Rossman's fluid (alcohol-formalin-picric acid) or Bouin's fluid, staining with H. & E., Masson's trichrome stain, or van Gieson's stain.

II. Histological Studies

(1) *Cytoplasmic basophilia*: fixation in Rossman's fluid, staining with 0.5% toluidine blue at pH 6.

(2) *Lipoids*. (a) Fixation in formal-calcium (Baker), gelatin embedding, frozen sections at 10 μ ; staining in saturated solution of a mixture of Sudan III and Sudan IV in 70% alcohol, or in a saturated solution of Sudan black B in 70% alcohol.

(b) Fixation in formal-calcium, staining by Baker's acid-haematein method (Baker, 1946), controlled by pyridine extraction.

(3) *Polysaccharides*. Fixation in Rossman's fluid, staining by the periodic-acid-Schiff method, controlled by diastase digestion.

(4) *Metachromatic substances*. Fixation in Bouin's or in Rossman's fluid, staining with 0.5% toluidine blue at pH 6 and pH 4.

(5) *Alkaline phosphatase*. Fixation in absolute acetone or in 80% alcohol at 0° C.; staining by the calcium phosphate method of Gomori (1952, p. 184) and by the azo-dye coupling method, using sodium α -naphthyl-phosphate as substrate (Gomori, 1952, p. 185).

III. Mitochondria

Fixation in Regaud's fluid, staining by the aniline-acid-fuchsin-methyl green method.

IV. Blood vessel injections

One carotid artery in a freshly killed adult female Aylesbury duck was cannulated, and the blood vessels washed out with normal saline and injected with undiluted indian ink. The nasal glands were removed, fixed in formalin, embedded in gelatin, and frozen sections were cut at 50 and 100 μ .

V. Functional studies

Two ducks were deprived of food (but not water) for 24 hr., and were then given salt by mouth (as Tab. NaCl, B.P.). Samples of nasal secretion and of liquid cloacal excreta were collected and their chloride content determined by titration against mercuric nitrate (Schales & Schales, 1941). In some samples, sodium and potassium were also estimated, by flame photometry, and in one of the birds, sodium and potassium were estimated in blood samples also.

RESULTS

I. Histological

The histological appearances are essentially as noted briefly in the duck by Marples (1932) and are described here in more detail to facilitate understanding of the histochemical findings. The gland, which is about 2.5 cm. long, is roughly triangular in cross-section, and is divided into lobules by septa of dense fibrous connective tissue which are continuous with the investing capsule (Pl. 1, fig. 1). The two main ducts extend almost through the entire length of the gland. At the anterior end of the gland (nearest to the nasal cavity), the ducts have a maximal external diameter of about 300 μ and are lined by a tall columnar epithelium, about 70 μ thick, in which the nuclei are disposed mainly in two layers, one basally placed, the other in the apical one-third of the epithelium. As they run posteriorly through the gland the ducts become slightly smaller, due to a reduction in the thickness of epithelium; the two-layered disposition of the nuclei is, however, maintained. Primary branches arise at obtuse angles from each of the main ducts; the frequency of branching may be judged from the fact that in a random 1 mm. length of gland, studied in serial sections, sixteen branches were counted. The primary branches, after a shorter or longer course, in turn divide. The terminal radicles of the duct system, which will be referred to as ductules, are lined by a simple, or occasionally two-layered, cubical epithelium (Pl. 1, fig. 2). From the ductules there arise the secretory tubules, which are blind-ending, fairly straight, simple, or branched tubes, lined by simple cubical epithelium, and possessing a minute lumen. Characteristically, the secretory tubules are arranged radially about a more or less centrally placed ductule, to constitute a lobule of the gland (Pl. 1, fig. 3). Secretory tubules are also found in groups opening directly into the main ducts, or into one side only of a short primary duct. Despite these varying connexions with the duct system, the secretory tubules appear uniform in structure throughout the gland. Since the lobule, with its centrally placed ductule and radiating secretory tubules, is a readily recognizable unit, all histochemical localizations will be referred to it.

In sections stained with haematoxylin and eosin, the most striking feature of the cells of the secretory tubules is the oesinophilia of their cytoplasm. This is so marked that in low-power view the central three-fourths or so of the lobule is stained bright pink. In contrast, the peripheral one-fourth of the lobule appears blue or purple, the reason for the difference being that the cells at the periphery have less cytoplasm (cf. Pl. 1, figs. 2, 4).

The secretory cells show also a curious granularity of the cytoplasm, which specific staining shows to be due to the presence of very abundant mitochondria (see later).

II. *Histochemical*

(a) *Toluidine blue*. The appearance of sections stained with toluidine blue at pH 6 is what might be anticipated from the H. & E. sections; in the central three-fourths of the lobule the cytoplasm is very palely stained, while the moderate basophilia of the periphery of the lobule is due very largely to nuclear staining. The absence of cytoplasmic basophilia in the secretory cells is taken to indicate virtual absence of cytoplasmic RNA.

Metachromatic staining is entirely absent in the secretory tubules in sections stained at pH 4 and 6; it may be noted, incidentally, that the efficacy of the dye and of the method used is illustrated by the presence of scattered mast cells, in which metachromatic staining is evident.

(b) *Periodic acid-Schiff*. The inter- and intralobular connective tissue, the walls of arterioles and venules, and reticular fibres surrounding the secretory tubules, are stained with moderate intensity in both untreated and diastase-treated controls.

The cytoplasm of cells of the secretory tubules is stained, but much less intensely than other positive elements. This faint staining is also seen in diastase-treated controls; it is not, therefore, due to glycogen.

(c) *Sudan III and IV, Sudan black*. Slides stained in a mixture of Sudan III and Sudan IV show a pale diffuse orange coloration of the epithelial elements of the gland, only slightly stronger than that of the interlobular connective tissue.

With Sudan black, on the other hand, the secretory tubules are stained quite intensely (Pl. 2, fig. 5). The epithelial nuclei are unstained and appear as pale circles against the black background of the cytoplasm. The peripheral blind ends of the secretory tubules stain less intensely than the central portion, partly because the nuclei are larger and the cytoplasm less abundant. In the cells of the central region, the staining appears fairly diffuse and uniform; only occasionally can discrete granules be recognized. Staining of the epithelium of the main ducts and of the ductules is generally weak.

(d) *Baker's acid haematein*. The distribution of blue-black coloration, specific for phospholipids when Baker's routine is followed closely, parallels that described for coloration by Sudan black (Pl. 3, figs. 6, 7). The reaction is absent in pyridine extracted controls.

(e) *Alkaline phosphatase*. The distribution of alkaline phosphatase shows some interesting differences in glands from immature and mature ducks. In glands from immature birds, positive staining is confirmed to the extreme periphery of the lobules, and, in the calcium phosphate preparations, is predominantly nuclear (Pl. 2, fig. 8; Pl. 3, fig. 9). The same general pattern of distribution is seen also in slides stained by the azo-dye coupling methods, but here the staining is not discrete enough to enable one to decide whether the enzyme is nuclear or cytoplasmic, or both. In glands from adult birds, on the other hand, the large interlobular vessels are intensely stained, and there are only scattered foci of staining in the secretory cells at the periphery of the lobules. As in the immature gland, the staining appears to be largely nuclear in distribution. There is no constant topographical relationship between the intensely stained interlobular blood vessels, on the one hand, and the scattered foci of staining of cells in the lobules on the other. Pl. 3, fig. 10,

illustrates the absence of staining in part of a lobule immediately adjacent to a (strongly positive) interlobular blood vessel, while Pl. 3, fig. 11, shows well-marked staining at the periphery of a lobule, with no other stained element in the vicinity. These findings speak against, although they do not definitely exclude, the possibility that the staining of peripheral cells of the lobule is a diffusion artefact.

It should perhaps be emphasized that neither the great bulk of secretory elements, nor the very abundant capillaries which run radially between the secretory tubules, show any staining with either method in glands from both young and mature ducks.

III. *Mitochondria*

Marples (1932) stained the mitochondria in glands from a number of species, and noted, almost as an incidental finding, that they are 'thickly and evenly scattered throughout the cytoplasm'. The statement scarcely does justice to the situation; in preparations stained by the acid fuchsin-methyl green method, mitochondria are so abundant that even in $3\ \mu$ sections studied under the low power, the central five-sixths or so of the lobule is stained uniformly and strongly pink, while the peripheral portion of the lobule is only palely stained.

These variations in intensity of staining reflect the distribution of mitochondria, which are most abundant in the cells of the inner five-sixths of the secretory tubules, i.e. towards the centre of each lobule. Here they occupy most of the available cytoplasmic space (Pl. 3, fig. 12; Pl. 4, fig. 13). They are so closely packed that it is difficult to be certain about their individual form, but they appear to be mainly spherical or ovoid, rather than filamentous. They are not arranged haphazardly within the cell, but in curious regular rows, each of which is separated from its neighbours by narrow unstained bands. This arrangement confers on the secretory cell a peculiar striped appearance. In general, these mitochondrial rows lie parallel to the longitudinal axis of the cell; a narrow zone at the apex of the cell is entirely free of mitochondria (Pl. 3, fig. 12).

At the peripheral blind ends of the secretory tubules, on the other hand, mitochondria are relatively sparse (Pl. 4, fig. 14). They are least abundant of all in cells of the duct system.

When sections stained with haematoxylin and eosin and by the PAS method were re-examined in the light of these observations on the mitochondria, it became evident that the ill-defined granularity of the cytoplasm noted in them was due to the extraordinarily abundant mitochondria.

IV. *Blood vessels*

The unusually rich vascularity of the gland is revealed in specimens injected with indian ink. Large vessels lie in the interlobular connective tissue; abundant intra-lobular capillaries surround the secretory tubules and, like them, are radially arranged (Pl. 4, figs. 15, 16).

V. *Secretory activity*

No secretion—other than the usual mucous film—is produced from the nasal cavity of ducks maintained on the laboratory diet of grain. Following the oral administration of sodium chloride, however, there occurs a copious and prolonged

secretion of a clear, watery fluid, which wells from the external nares and, unless the bird is restrained, is repeatedly shaken from the bill. This extraordinary response is the same as that described by Schmidt-Nielsen *et al.* in the cormorant and penguin.

Details of the response in two ducks are summarized in Table 1.

Table 1

Duck 4/8. Weight = 3.1 kg. Dose of NaCl = 4 g. (in 50 ml. water). Interval between administration and onset of secretion = 45 min.

Collection period (each of 30 min.)	Volume of secretion (ml.)	Chloride (m-equiv./l.)	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)
1	3.8	462	—	—
2	6.5	495	408	14
3	8.0	517	—	—
4	7.5	539	408	15
5	6.5	495	—	—
6	6.5	528	408	14
7	6.0	528	—	—

Total time of collection	= 3½ hr.
Total volume of nasal secretion	= 44.8 ml.
Total nasal output of chloride	= 0.7 g. (approx.)
Total volume of urine (3½ hr.)	= 16 ml.
Chloride content	= 110 m-equiv./l.
Total urinary output of chloride	= 0.06 g. (approx.)
Total input of chloride	= 2.43 g. (approx.)

Duck 6/8. Weight = 3.6 kg. Dose of NaCl = 4.7 g. (in 35 ml. water). Interval between administration and onset of secretion = 15 min.

Collection period (each of 30 min.)	Volume of secretion (ml.)	Chloride (m-equiv./l.)	Sodium (m-equiv./l.)	Potassium (m-equiv./l.)
1	2.5	490	—	—
2	8.0	497	—	—
3	10.0	522	435	15.5
4	10.0	492	—	—
5	12.0	500	—	—
6	10.5	507	—	—
7	10.0	510	420	15
8	13.0	521	—	—
9	10.0	521	—	—
10	11.0	454	—	—
11	7.0	466	—	—
12	7.0	478	—	—

Total time of collection	= 6 hr.
Total volume of nasal secretion	= 111.0 ml.
Total nasal output of chloride	= 1.96 g.
Total volume of urine (6 hr.)	= 14 ml.
Chloride content	= negligible
Total urinary output of chloride	= negligible
Total input of chloride	= 2.85 g. (approx.)

Blood samples.

A. Collected during collection period (3) { Sodium = 111 m-equiv./l.
Potassium = 3 m-equiv./l.

B. Collected during collection period (7) { Sodium = 152 m-equiv./l.
Potassium = 3.9 m-equiv./l.

Qualitative tests were applied to the fluid for the presence of protein, mucin, and magnesium and ammonium ions; all were negative.

It may be of value, at this stage, to summarize the results of these functional studies on the duck, and to compare them with those already described in the

cormorant and penguin by Schmidt-Nielsen and co-workers. In duck 4/8, during a 3½ hr. collection period, about 30% of the ingested chloride was recovered from the nasal secretion, and only about 2.5% from the urine. In duck 6/8, about 70% of the ingested chloride was recovered from the nasal secretion during a 6 hr. collection period, and none from the urine. In the cormorant (Schmidt-Nielsen *et al.* 1958), excretion of chloride seems to be about equally divided between the nasal secretion and the urine, while in the penguin (Schmidt-Nielsen & Sladen, 1958) about 66% of the administered chloride was recovered from the nasal secretion during a 4 hr. collection period, and about 6% from the urine. The electrolyte concentrations, and the volume of secretion produced are of the same order of magnitude in all three species.

Comparison of the concentration of sodium and potassium in the serum and in the nasal secretion in the duck emphasizes the great metabolic activity of the glands responsible. These electrolytes—and presumably chloride also, although this was not measured in the serum—are concentrated about 4 times by the secretory cells.

DISCUSSION

These findings reveal the nasal gland of the duck as a richly vascular, lobulated compound tubular gland, with some most unusual and interesting histological features.

It is not a mucus-secreting gland. Not only does it not look like one in ordinary histological sections, but the histochemical tests for mucous elements—the PAS stain, and metachromatic staining with toluidine blue—are both negative.

Neither does it possess the usual characteristics of a 'serous' gland, using that term to indicate a gland which elaborates a watery, enzyme-rich secretion. There is no evidence of 'zymogen' granules, and the secretory cells do not show the cytoplasmic basophilia which one associates with serozymogenic cells.

The very weak coloration with the mixture of Sudan III and IV, compared with the well-marked coloration with Sudan black, strongly suggested the presence of phospholipid in the secretory tubules, and this was fully established by the positive reaction to Baker's acid haematein test, controlled by pyridine extraction.

The distribution of phospholipid parallels that of mitochondria: sparse in the main ducts, in the ductules, and at the blind ends of the secretory tubules, and abundant in the remaining cells of the secretory tubules. This coincidence of distribution suggests that the phospholipid might in fact be mainly mitochondrial. Mitochondria are known to contain much phospholipid and the acid-fuchsin-methyl green preparations show an extraordinary abundance of mitochondria. They are so closely packed, even in a 3 μ section, that one is not surprised to find dense and relatively uniform staining with Sudan black and with the acid haematein method in 10 μ frozen sections. The staining of mitochondria in some types of cells by the acid-haematein method was shown by Baker (1946) in his original description of the technique, which in fact provided the first rigorous histochemical proof that mitochondria contain phospholipid.

The study of alkaline phosphatase in the gland has so far yielded results of rather doubtful significance. All that one can say at present is that the enzyme is probably not directly related to the specific functional activity of the gland. This is indicated

by the absence of enzyme both from the great bulk of secretory cells of the gland, and from endothelial cells of the intralobular capillaries.

These histological, cytological, and histochemical findings—and indeed the facts of the comparative morphology of the gland—become significant and understandable in the light of the physiological findings of Schmidt-Nielsen *et al.* (1958) and Schmidt-Nielsen & Sladen (1958). In the cormorant and penguin, excess salt intake provokes the secretion into the nose of substantial quantities of hypertonic sodium chloride solution. The present study has shown that the same is true of the duck.

Of all the sites within or about the nose from which such a nasal secretion might arise, the nasal gland seems most fully to satisfy the histological desiderata. It is unusually vascular, as one might expect of a gland which can produce about 110 ml. of watery fluid in 6 hr. Its cells contain abundant mitochondria, which in many other situations have been shown to contain many of the cellular enzyme systems, and whose number is generally thought to be related to the metabolic activity of the cell. A high rate of metabolism—and abundant mitochondria—might be confidently expected of a cell which can elaborate a secretion containing a concentration of sodium and potassium—and presumably of chloride also—some four times greater than that in the serum. The negative findings are also important: the absence of mucus, and of protein, from the secretion correlates with the absence of mucous and serozymogenic elements in the gland.

Finally, the secretory cells of the nasal gland may be very profitably compared, structurally and functionally, with two cell types generally believed to be involved in the active secretion of chloride ions—the oxyntic cell of mammalian gastric mucosa, and the 'chloride cell' of the gills of euryhaline fishes.

The eosinophilia of the oxyntic cell is well known and matches that of the nasal gland cell; with methyl green-pyronin staining, Menzies (1952) failed to detect any cytoplasmic basophilia in the oxyntic cell. The 'granules' which he showed histochemically (Menzies, 1949) to contain a lipid and a phospholipid component are now certainly known to be mitochondria; their abundance is equalled, if not excelled by, that in the nasal gland cell. Alkaline phosphatase is absent from both types of cell.

Active excretion of salt from the head region of marine teleosts was first shown by Homer Smith (1930), and the locus of activity was shown to be the gills by Keys (1931), Bateman & Keys (1932), and Schlieper (1933). The particular cells involved were suggested by Keys & Willmer (1932) to be large, rounded and conspicuously eosinophilic cells on the gill filaments, and although this suggestion was disputed by Beverlander (1936), the histochemical localization of chloride within the cell by Copeland (1948) clinched the matter. Later, Copeland (1950) characterized the cells as eosinophilic, osmiophilic, and rich in mitochondria; when active, they were shown to contain alkaline phosphatase. In another paper, Burns & Copeland (1950) showed that in *Fundulus* the chloride-excreting cells are not confined to the gills but are distributed throughout the epithelium of the head cavity. Of particular interest in the present context is their observation that the cells are much more abundant in regions of rich vascularity.

It will be seen that there are many resemblances between the nasal gland cell, the oxyntic cell, and the Keys-Willmer cell of fish gills. These resemblances are the more

striking if, as seems likely, these cells are responsible for active secretion of chloride ions.

Some comment must be made on the observation of Marples (1932) that the ductules and excretory ducts in the nasal glands of all the species he has studied contained cellular debris, and on his surmise that the secretion is the product of disintegrating cells and that the glands are holocrine in type. In the inactive gland of the duck I have seen no signs of cellular debris within the duct system; moreover, in a preliminary study of sections of actively secreting glands, cellular debris within the ductules and main ducts has been an uncommon finding, and it seems most unlikely that it is the source of the secretion.

Further work is necessary to establish fully the functional significance of the nasal glands of birds. The findings of the present study provide strong anatomical support for the view that they are responsible for the extraordinary secretory response to salt loading. If this view should prove to be well founded, the activity of the glands might have an important bearing on the problem of water balance in marine birds, some of which spend months at sea without access to fresh water. There is little information available about water-balance in marine birds *per se*, but other members of the avian Class are known to possess a number of important water-conserving mechanisms. First, the Malpighian corpuscles of the avian kidney are poorly vascularized (Marshall & Smith, 1930), and this might be assumed to dictate a low rate of filtration (clearance studies, however, throw some doubt on the validity of this assumption; see Sturkie (1954) for discussion). Secondly, uric acid rather than urea is the end-point of protein metabolism in birds; this requires less water than does urea to rid the body of the nitrogen residue from a given quantity of protein. Thirdly, in some species there is said to occur cloacal or rectal reabsorption of water (Sturkie, 1954, p. 221). Fourthly, birds have no sweat glands, and are therefore spared the large water-loss in which these involve man and some other mammals. (As against this, their air sacs provide an additional site of water-loss (Sturkie, 1954, p. 105).)

These four water-conserving mechanisms could make possible, in marine birds, a relatively small water intake, which might be provided by metabolic water and, in fish eaters, by the tissue juices of their prey. Excess salt intake either in the food or in swallowed sea water can be eliminated in the nasal secretion which, being more concentrated than sea water, allows a net saving of water (see Schmidt-Nielsen *et al.* (1958) for fuller discussion of this aspect of the problem).

The nasal glands are of less obvious value in the domestic duck whose usual habitat provides an abundance of fresh water. One can only assume that its nasal glands are normally seldom active and that they are more immediately useful to its wild ancestor, the mallard, from which it is little removed, and which is equally at home on river and estuary, pond and sea-shore.

SUMMARY

1. The histological structure and some aspects of the histochemistry and functional activity of the nasal (supra-orbital) glands have been investigated in the domestic duck.

2. The gland is a compound tubular one, and is highly vascular.

3. The secretory cells of the gland are characterized by their eosinophilia and by the presence of unusually abundant mitochondria. They are rich in lipid stainable by Sudan black, much of which appears to be phospholipid and to belong to the mitochondria.

4. Serozymogenic and mucous elements are absent from the gland.

5. Excess dietary intake of sodium chloride provokes the secretion into the nasal cavity of a clear, protein free, watery fluid, containing about 3% sodium chloride.

6. The histological and histochemical evidence is consistent with the view that this secretion is elaborated by the nasal glands.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. General view of nasal gland, in transverse section, to illustrate its lobular character and the presence of two main ducts. H. & E. $\times 15$.
- Fig. 2. A higher power view of part of the central region of a lobule, showing, above, a ductule into which opens, below, a secretory tubule. Masson. $\times 420$.
- Fig. 3. General view of a single lobule. Centrally, cut transversely, is the ductule, from which radiate the secretory tubules. Note: the 'holes' at the periphery of the lobule (at 11, 1 and 4 o'clock) are histological artefacts. H. & E. $\times 150$.
- Fig. 4. A higher power view of the periphery of part of a lobule. The centre of this lobule lies (out of the field) above and to the left. Note that the nuclei are more abundant towards the peripheral blind ends of the secretory tubules and that the cytoplasmic volume is proportionately less. Masson. $\times 420$.

PLATE 2

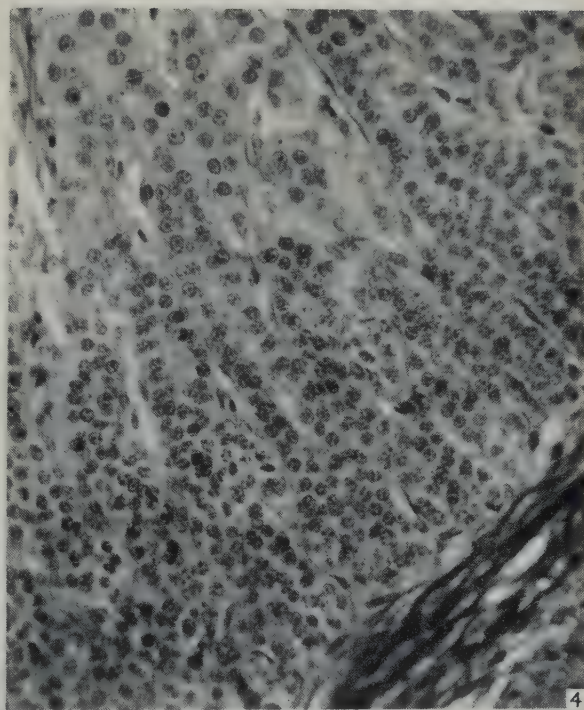
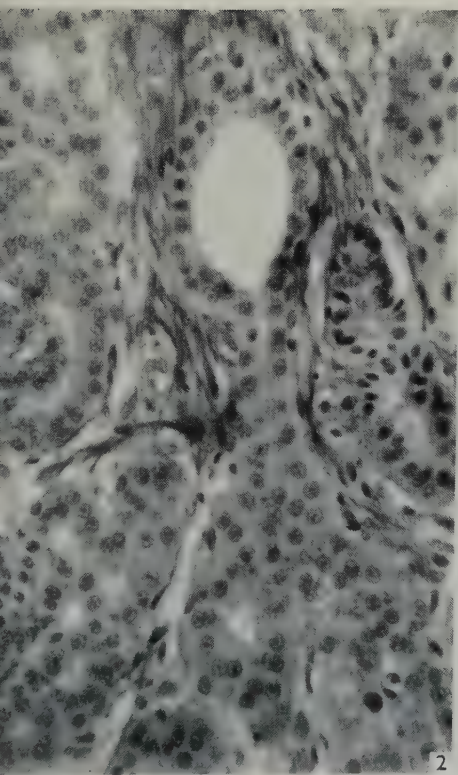
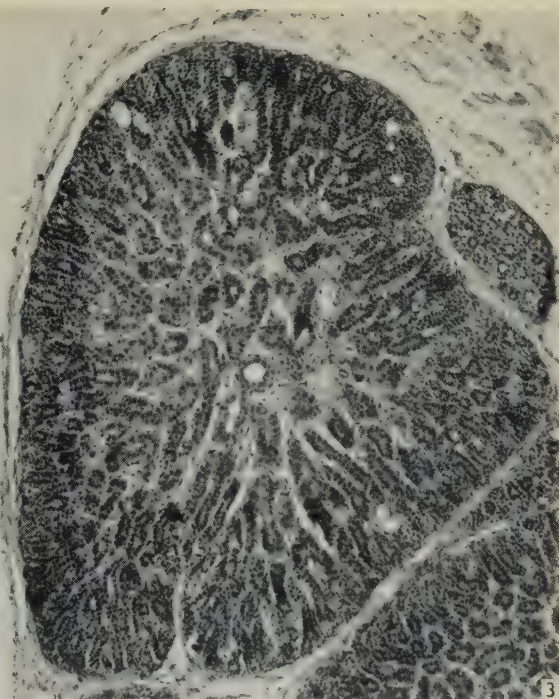
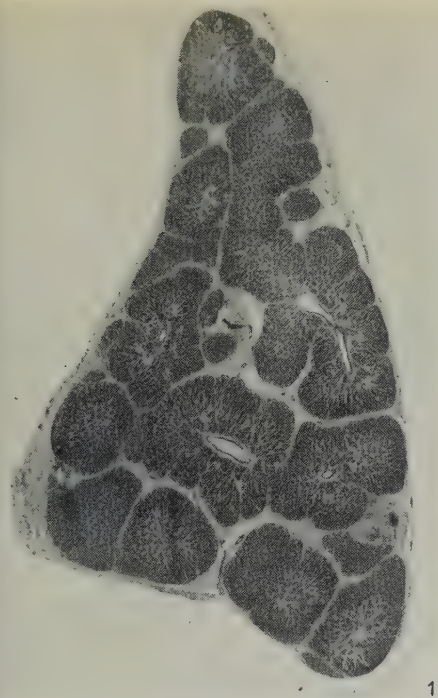
- Fig. 5. General view of lobule in transverse section, to show distribution of lipid. Formal-calcium fixation, frozen section, Sudan black. $\times 90$.
- Fig. 6. General view of lobule in transverse section, to show distribution of phospholipid. Baker's acid-haematein method. $\times 90$.
- Fig. 7. Higher power view of fig. 6, to show abundance of phospholipid in the cells of the secretory tubules, except at their blind peripheral ends. Baker's acid-haematein method. $\times 750$.
- Fig. 8. To illustrate the distribution of alkaline phosphatase in nasal gland from an immature duck. An interlobular blood vessel is stained, but a positive reaction is otherwise confined to cells at the extreme periphery of each lobule. Gomori's calcium phosphate method, 3 hr. incubation, 15μ section. $\times 180$.

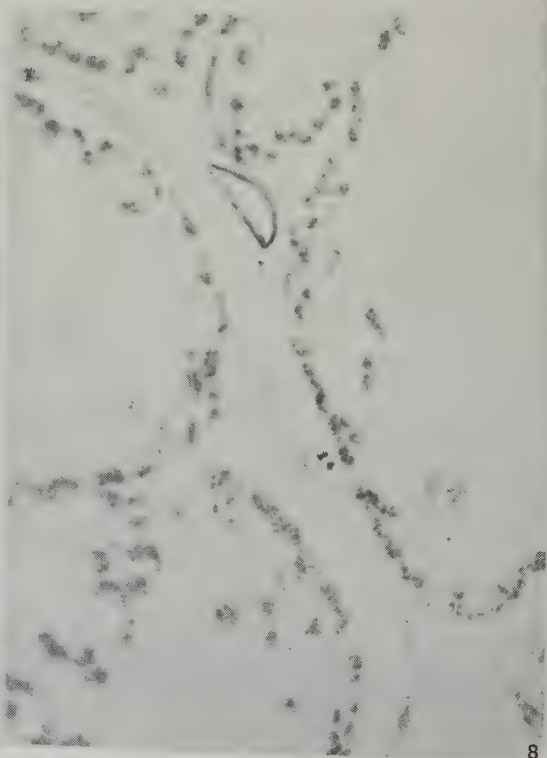
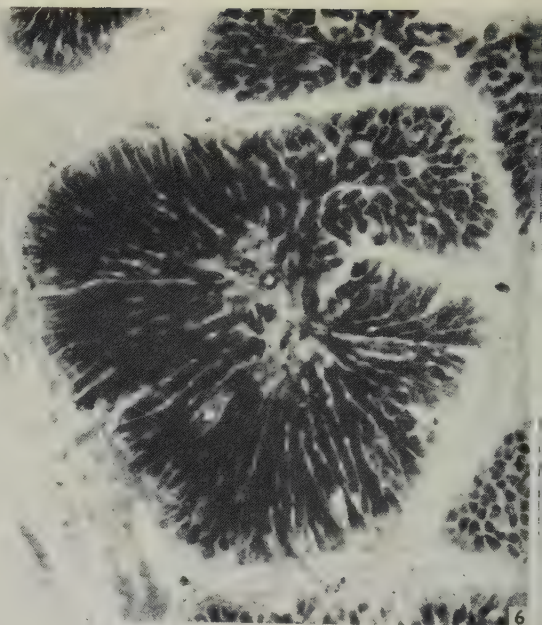
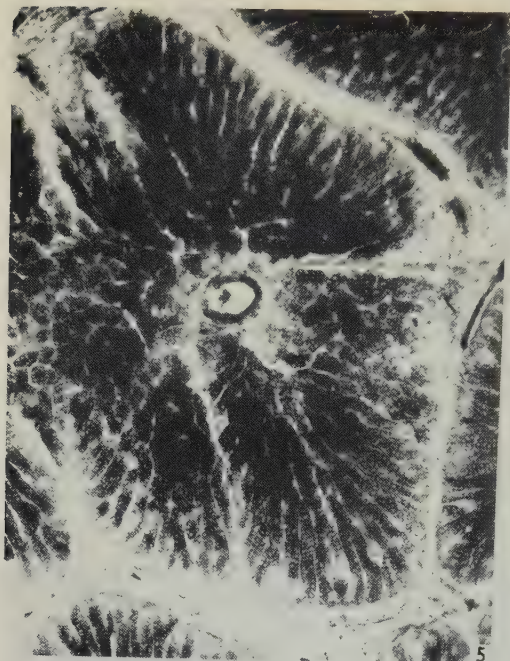
PLATE 3

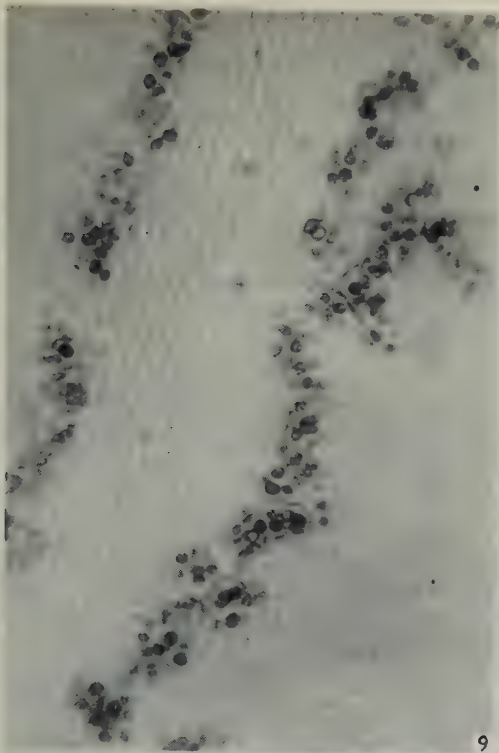
- Fig. 9. A higher power view of fig. 8, to show that the alkaline phosphatase is apparently largely nuclear in distribution. $\times 420$.
- Fig. 10. The distribution of alkaline phosphatase in a gland from an adult duck. Centrally is a strongly positive interlobular blood vessel, to the right lies the periphery of part of a lobule. Note absence of staining of secretory elements. 10μ section. Gomori's calcium phosphate method, $1\frac{1}{2}$ hr. incubation. $\times 450$.
- Fig. 11. From same section as fig. 10, showing patchy foci of alkaline phosphatase activity at the periphery of a lobule. Note the absence of any other positively stained structure in the vicinity. $\times 450$.
- Fig. 12. Shows, centrally, a transverse section of a secretory tubule. Mitochondria appear black, nuclei as pale areas. Note great abundance of mitochondria, except in a narrow zone at the apex of the cell where they are virtually absent. Aniline-acid fuchsin-methyl green. 3μ section. Oil immersion.

PLATE 4

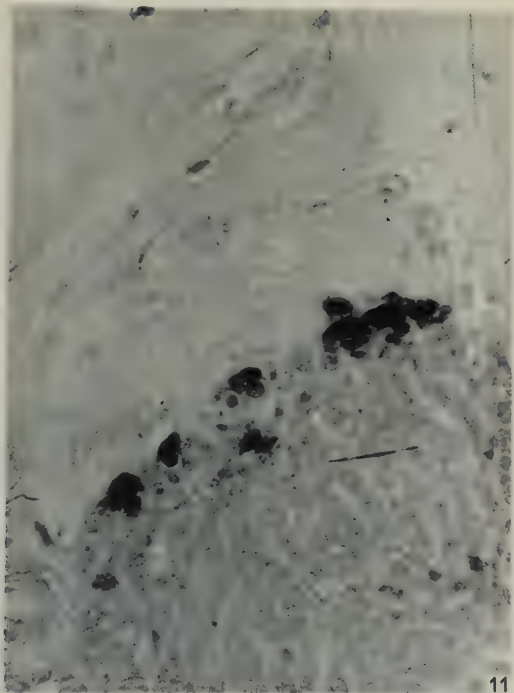
- Fig. 13. Part of a secretory tubule in longitudinal section. The lumen of the tubule is seen at about the centre of the photograph; below and to the left of the lumen, five individual mitochondria may be recognized. Elsewhere, note the dense packing of mitochondria in horizontal rows, giving the secretory cells a striped appearance. Aniline-acid-fuchsin-methyl green. 3μ section. Oil immersion.
- Fig. 14. In longitudinal section, the peripheral blind end of a secretory tubule. Note that mitochondria are less abundant than in cells of remainder of tubule (cf. figs. 12-14), that they again show a linear arrangement, and that they are absent from a narrow strip at the apex of each cell. Aniline-acid-fuchsin-methyl green. 3μ section. Oil immersion.
- Fig. 15. Thick (100μ) section of gland, to show blood vessels, injected with indian ink. $\times 18$.
- Fig. 16. Higher power view of fig. 15, showing the radially arranged capillaries of a single lobule. $\times 120$.



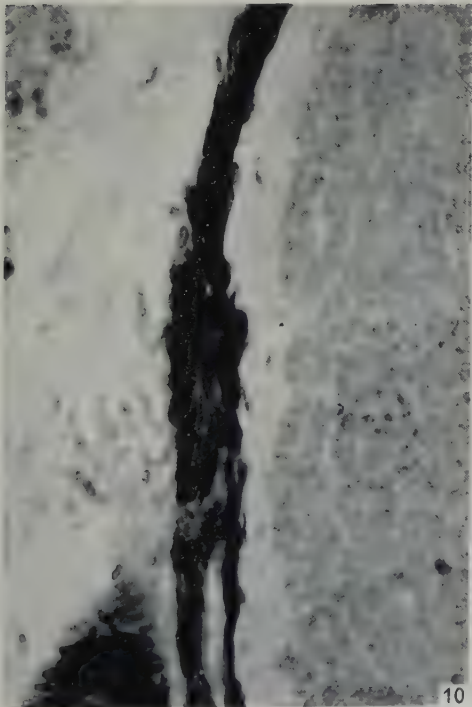




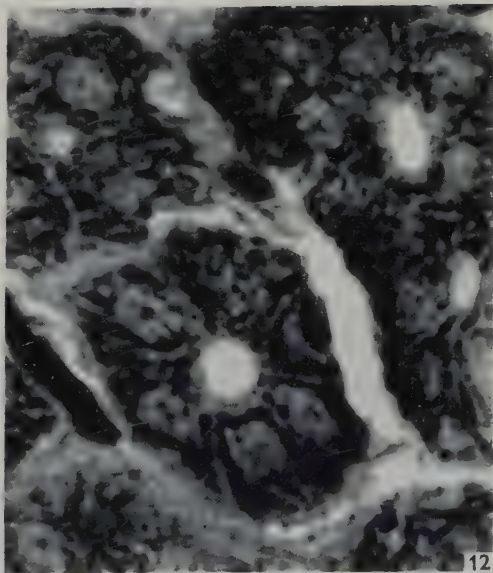
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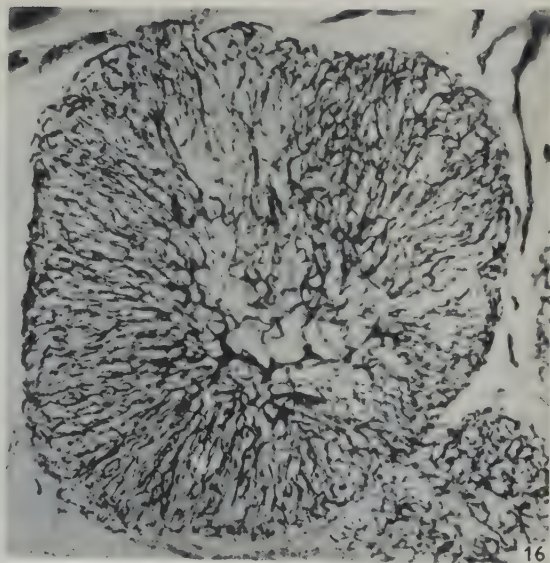
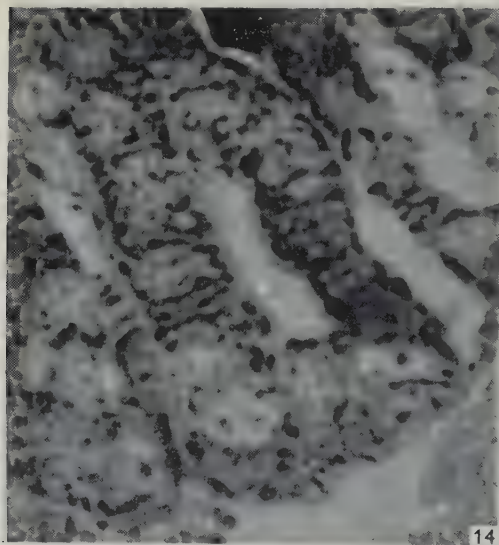
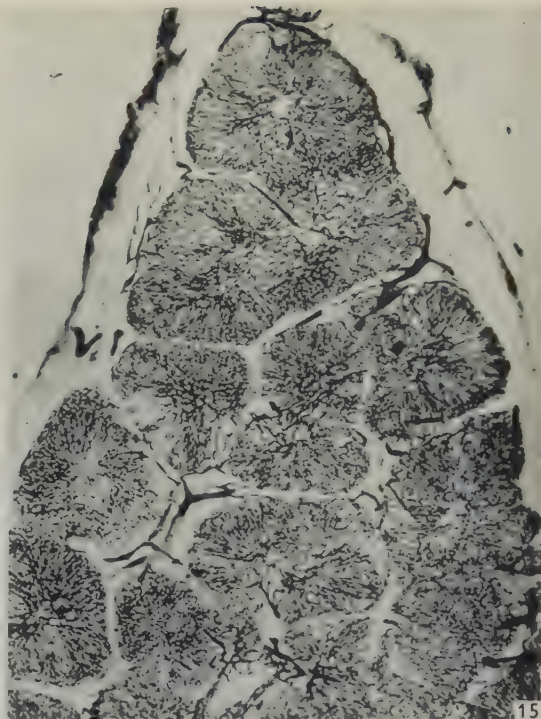
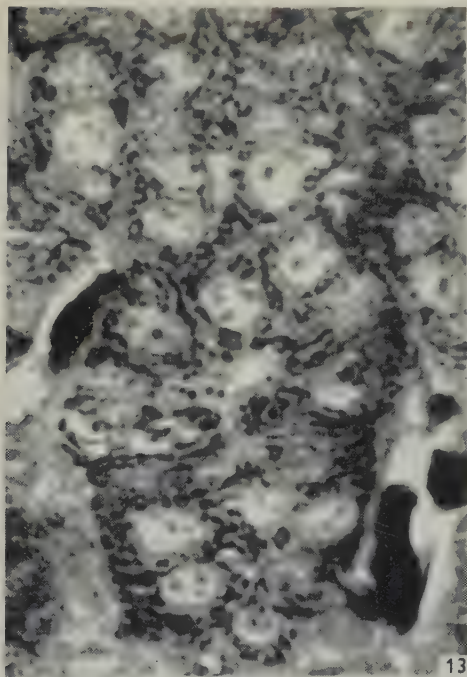
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SCOTHORNE—NASAL GLAND IN THE DOMESTIC DUCK

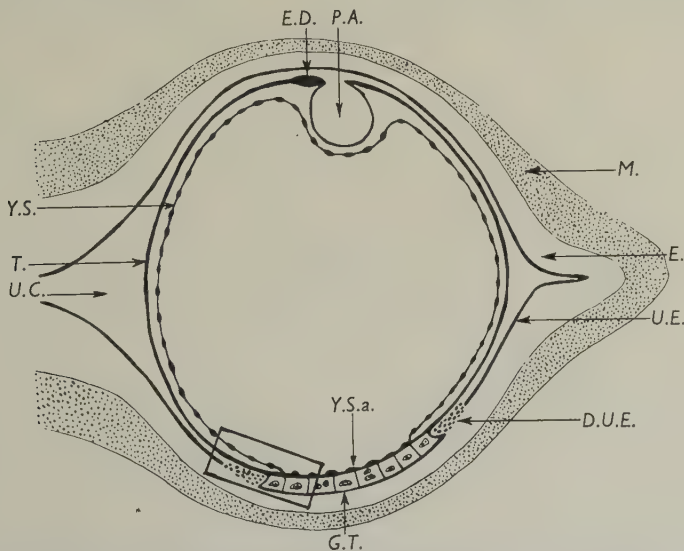
AN EARLY BLASTOCYST OF THE LESSER BUSH BABY (*GALAGO SENEGALENSIS SENEGALENSIS*); A PRELIMINARY ACCOUNT

By H. BUTLER

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Preliminary field observations (Butler, 1957) indicated that, in the province of Kordofan, early pregnancy in the Lesser Bush Baby (*Galago senegalensis senegalensis*) should be encountered in late November or early December. A further field expedition, made in early December 1957, collected eight uteri and six of these contained early pregnancies. All the uteri were fixed in Bouin's fluid within an hour or less of death. So far, one pregnant uterus has been serially sectioned at 5μ and it shows a blastocyst with a most unusual mode of implantation.

The pregnant horn of the uterus (measured after fixation) is approximately 9.0 mm. long and has a maximum diameter of 5.0 mm.; the non-pregnant horn is slightly less than half this size. A graphic reconstruction (Text-fig. 1) shows that



Text-fig. 1. Reconstructed longitudinal section of the uterine horn and blastocyst. Photomicrographs of the area enclosed in the rectangle are shown in Pl. 1, figs. 1-4.

Abbreviations used in Text-fig. 1

<i>D.U.E.</i>	Degenerating uterine epithelium	<i>T.</i>	Small cell trophoblast
<i>E.</i>	Endometrial stroma	<i>U.C.</i>	Uterine cavity
<i>E.D.</i>	Embryonic disc	<i>U.E.</i>	Uterine epithelium
<i>G.T.</i>	Giant cell trophoblast	<i>Y.S.</i>	Free portion of yolk sac
<i>M.</i>	Myometrium	<i>Y.S.a.</i>	Attached portion of yolk sac
<i>P.A.</i>	Pro-amnion		

the uterine distension is due to the presence of a large, almost spherical, centrally implanted blastocyst, some 4.5 mm. in diameter. The blastocyst is, for the most part, bilaminar consisting of an outer trophoblastic shell surrounding a capacious yolk sac; some extra-embryonic mesoderm is present in certain localities. An embryonic disc, 220 μ long, is situated at the anti-mesometrical pole of the blastocyst and here the ectoderm is invaginated to form a pro-amnion.

The greater part of the trophoblast consists of a single layer of cuboidal cells (averaging 10 μ thick) lying in intimate apposition to apparently unaltered uterine epithelium. Where the trophoblast has been pulled away from the uterine epithelium it is apparent that its maternal surface is moulded to that of the uterine epithelium. Cytoplasmic processes of the trophoblast cells, which appear spike-like in section, penetrate in between adjacent uterine epithelial cells (Pl. 1, fig. 1). The uterine horn contains eighty compound uterine glands and fifty-six of these are overlaid by this part of the trophoblast. Over each gland mouth the trophoblast cells are enlarged and distended by large vacuoles and appear to be actively absorbing glandular secretion (Pl. 2, fig. 5). These specialized absorptive areas are the precursors of the chorionic vesicles of the mature placenta.

The unique feature of this blastocyst is found upon its mesometrical aspect. Here, over an area measuring 2.5 by 4.0 mm., the trophoblast forms a single layer of giant cells (Pl. 1, figs. 2-4). This layer is 40 μ thick and is almost a syncytium, since the adjacent cell boundaries are frequently indistinct. The nuclei in this layer are mainly ovoid in shape, measuring 15 by 25 μ . In this area the uterine epithelium has completely disappeared and the giant cell trophoblast is in contact with the uterine stroma. Maternal capillaries are in close contact with the trophoblast and may, on occasion, groove its outer (maternal) surface. A few small haemorrhages are seen between the maternal and foetal tissues. Around the periphery of the giant cell area is a transitional zone in which the small cuboidal trophoblast cells are proliferating and metamorphosing into giant cells (Pl. 1, figs. 2, 3). The uterine epithelium cells end abruptly at the outer edge of the giant cell trophoblast (Pls. 1, 2, figs. 2, 3 and 6). A zone of altered uterine epithelium, some 200 μ wide, surrounds the giant cell area. Here the uterine epithelium cells are undergoing proliferation and degeneration. These changes are most marked immediately adjacent to the giant cell trophoblast and the nuclei may form three or four layers. This zone gradually gives way to normal uterine epithelium (Pls. 1, 2, figs. 1, 2 and 6). In the region of proliferation the cell outlines are indistinct and the condition is like the syncytial degeneration described in certain rodents by Mossman (1937). Small desquamating masses of degenerating uterine epithelial cells are apparently being absorbed by the trophoblast cells of the transitional zone, together with what appears to be uterine milk (Pls. 1, 2, figs. 2, 6). The edge of the giant cell trophoblast is undermining the degenerating uterine epithelium and spreading still further. The uterine stroma underlying the giant cell area is more oedematous than elsewhere. Only one uterine gland is completely overlaid by the giant cell trophoblast, but two others appear as if they are about to be covered. The uterine epithelium is absent right up to the edge of the gland mouth, but the epithelium of the gland neck appears unaltered and the trophoblast does not extend into the gland lumen. The trophoblast over this gland is no different from the remainder of the giant cell trophoblast.

The yolk sac is formed from a single layer of flattened and irregularly shaped endoderm cells which, for the most part, loosely lines the trophoblastic shell. In some places there are fragments of a hyaline membrane between the endoderm and trophoblast. This is regarded as the equivalent of Reichert's membrane, so frequently found in this situation in rodent blastocysts (Mossman, 1937). Over the inner surface of the giant cell trophoblast the endoderm cells are so markedly flattened that they resemble endothelium, and this part of the yolk sac is very firmly attached to the inner surface of the giant cell trophoblast, except at the circumferential transitional zone (Pl. 1, fig. 4). Reichert's membrane is absent in this area of yolk sac attachment. The endoderm adjacent to the ring of transitional trophoblast is covered by a narrow annular zone of extra-embryonic mesoderm. Between the endoderm and mesoderm are large fluid filled spaces (Pls. 1, 2, figs. 3, 7). Isolated areas of extra-embryonic mesoderm are scattered over the rest of the yolk sac. As yet, none of the extra-embryonic mesoderm exhibits vasculogenesis.

DISCUSSION

The unique feature of this blastocyst is the area of giant-cell trophoblast in direct contact with maternal connective tissue and capillaries. This relationship is totally unexpected in view of the fact that the mature placenta is generally regarded as being of the epithelio-chorial variety in all investigated forms of Strepsirhini (Hill & Burne (1922, *Chiromys*); Hill, Ince & Subbu Rau (1928, *Loris*); Hill (1932, *Loris*, *Nycticebus*)). The only exception being *Galago demidovii* which, according to Gérard (1929, 1932), shows an area of endothelio-chorial placenta surrounded on all sides by a diffuse epithelio-chorial arrangement. Giant cells, thought to be of trophoblastic origin, are found in the region of endothelio-chorial placentation.

The nearest comparable Strepsirhine blastocyst is that of *Loris* 5 described by Hill (1932). This specimen is 3.0 mm. in diameter and fills the uterine cavity. It is bilaminar, with a small embryonic disc, and the trophoblast, like that of the present specimen, exhibits spike-like processes penetrating between uterine epithelial cells and pro-chorionic vesicles. At the abembryonic pole Hill figures a specialized area of the trophoblast which he calls the 'absorption area' (see his Fig. 12, plate 1) where the trophoblast cells are enlarged and vacuolated. The uterine epithelium, however, is intact in this region. A partially sectioned blastocyst of *Galago senegalensis*, younger than the one herein described, shows similar trophoblast, and this appears to mark the commencement of the giant cell trophoblast. The blastocyst of *Nycticebus* 190 (Hill, 1932) apparently does not yet show any comparable trophoblastic modifications, presumably because it is younger. It is, however, of interest to note that Hubrecht (1908) stated that the conceptus of *Nycticebus* could be easily washed out of the uterus in the latter half of pregnancy but not so the blastocyst of 5.0–11.0 mm. This he attributed to the distension of the blastocyst, but it might well be due to a mode of attachment similar to that described above.

For the most part the blastocyst is bilaminar but extra-embryonic mesoderm is present in some areas. As yet, no vasculogenesis is present. In the region of attachment the blastocyst wall consists only of endoderm and trophoblast and so forms a metrioplacenta which Flynn (1923) defined as being fusion or intimate apposition of a bilaminar omphalopleure with the uterine wall.

One very striking feature of the blastocyst is the trophoblastic modifications which are clearly related to the extreme distension of the blastocyst (Pl. 2, figs. 5-7). These include fifty-six pro-chorionic vesicles and the area of giant cell trophoblast which performs the dual function of attachment and absorption.

SUMMARY

A preliminary examination of the earliest recorded blastocyst of the Lesser Bush Baby shows remarkable modifications of the trophoblast. There is a large area of giant cell trophoblast which is in direct contact with the endometrial stroma. This area of the blastocyst wall forms a bilaminar omphalopleure and so a metrio-placenta is present at this stage. Additionally, there are fifty-six pro-chorionic vesicles related to the uterine glands. Small amounts of extra-embryonic mesoderm, not yet exhibiting vasculogenesis, are present in some places.

I wish to record my thanks to Dr Mohamed Zaki Mustapha, Province Medical Officer of Kordofan, without whose invaluable help this unique material could not have been obtained. Also to Mr S. J. Woods for preparing the sections and assisting with the photography.

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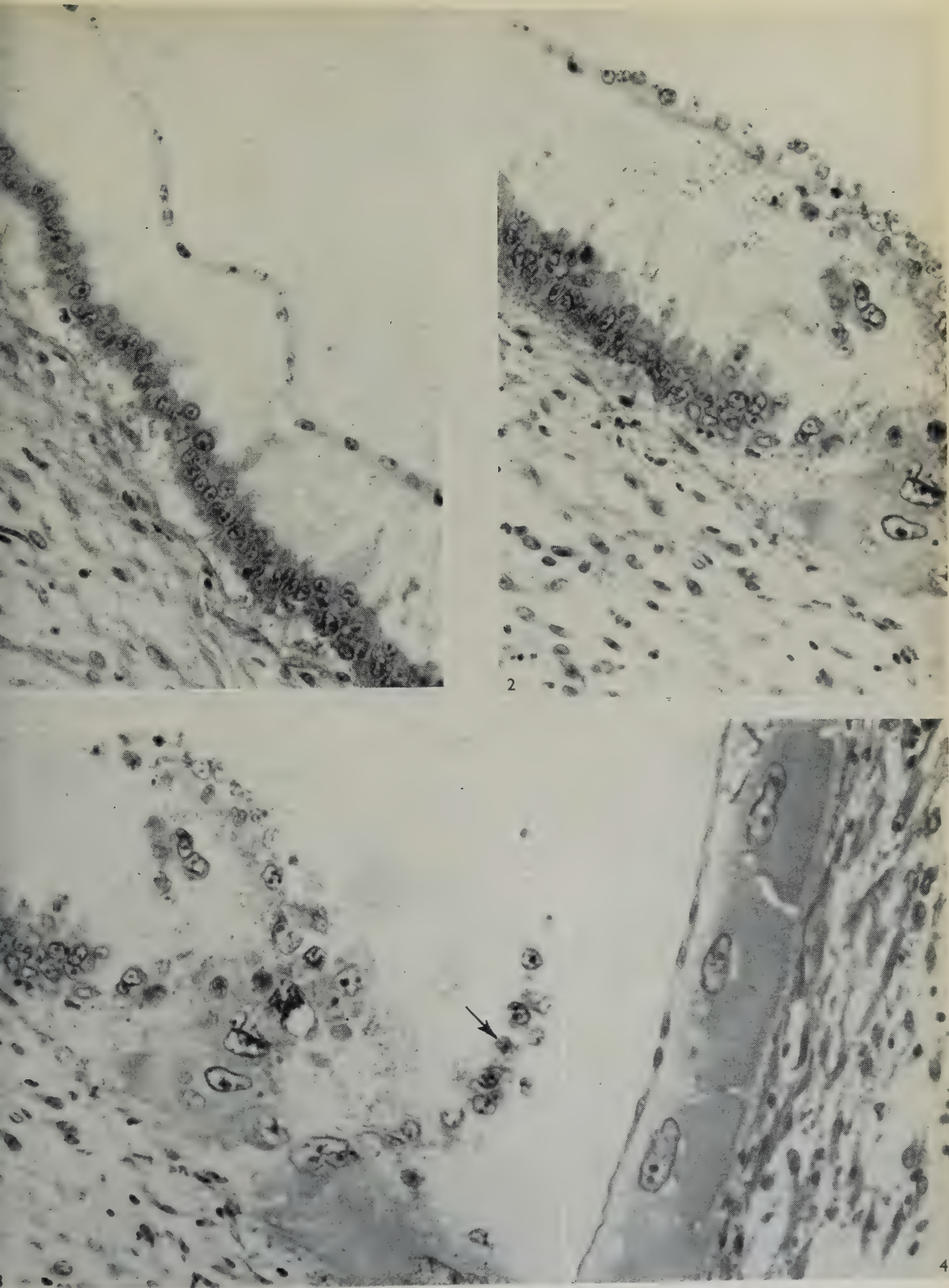
EXPLANATION OF PLATES

PLATE 1

Four views of a section through the area enclosed in the rectangle in Text-fig. 1, commencing at the extreme left. Stained iron haematoxylin and orange G, all $\times 400$.

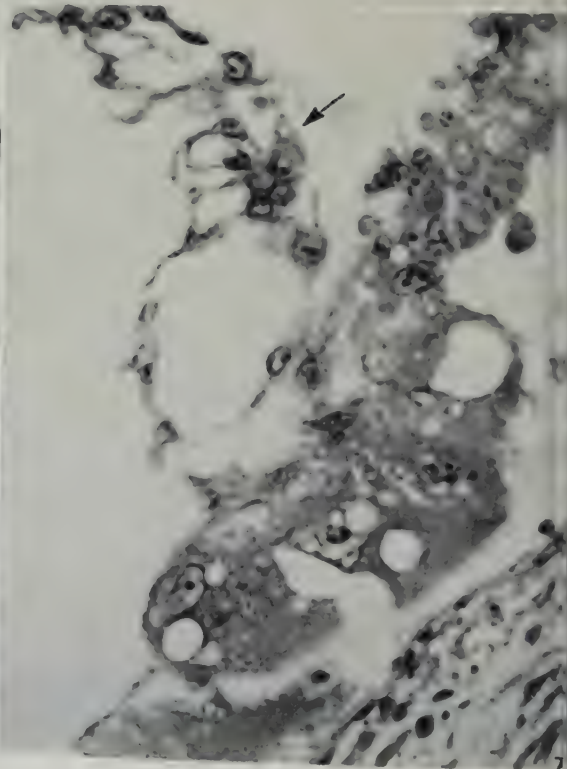
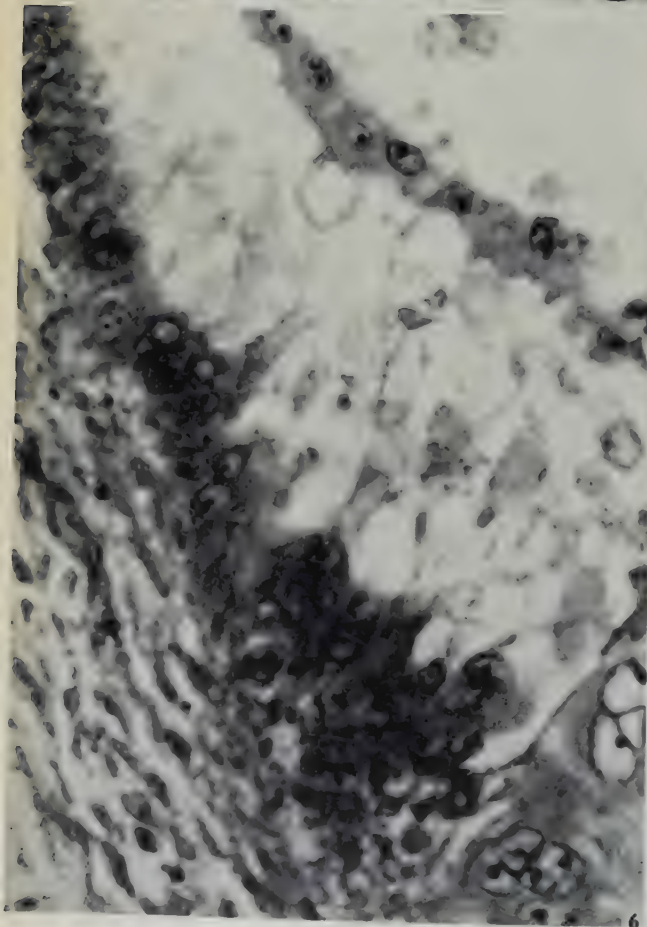
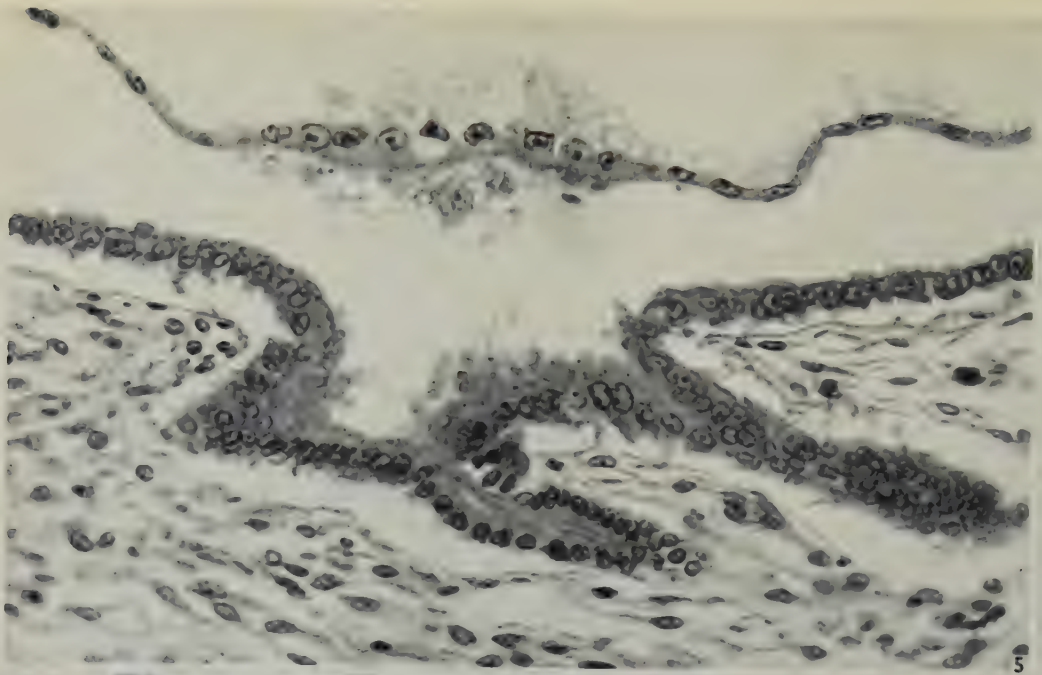
Fig. 1. Uterine epithelium and small cell trophoblast at the periphery of the zone of syncytial degeneration of the uterine epithelium. Above and to the left is unaltered uterine epithelium showing a single layer of nuclei. Below, and to the right, this epithelium merges gradually into the zone of syncytial degeneration. Separation of the trophoblast and uterine epithelium is an artefact of preparation.

Fig. 2. The same section, closer to the margin of the giant cell trophoblast, to show increased syncytial degeneration and the abrupt cessation of the uterine epithelium at the margin of the giant cell trophoblast. Note the group of desquamated uterine epithelial cells. The small cell trophoblast is thicker and its nuclei increase in number as it dips down to continue as the giant cell trophoblast. The latter is in direct contact with the endometrial stroma.



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(Facing p. 260)



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Fig. 3. A further view of the same section showing the transitional zone between the small and giant cell trophoblast. The trophoblast nuclei increase in size and number in this zone. To the right is the yolk sac wall as it makes contact with the giant cell trophoblast. The yolk sac wall is here two cells thick and the outer cells, i.e. those adjacent to the trophoblast, are regarded as extra-embryonic mesoderm (arrowed).

Fig. 4. Typical giant cell trophoblast in intimate contact with the endometrial stroma and, on its inner aspect, the single layer of firmly attached, endothelial-like endoderm cells. This is the region of the metrioplacenta.

PLATE 2

Sections of the trophoblast and yolk sac to illustrate the intense absorptive activity of these structures.

Fig. 5. Modified small cell trophoblast overlying the mouth of a uterine gland and forming a prochorionic vesicle. Haematoxylin and eosin. $\times 400$.

Fig. 6. A more highly magnified view of the junction of the uterine epithelium and giant cell trophoblast showing detail of the syncytial degeneration of the uterine epithelium and the absorption of degeneration products. Note also the groups of nuclei in the giant cell trophoblast. Iron haematoxylin and orange G. $\times 770$.

Fig. 7. The yolk sac wall, adjacent to the transitional zone of the trophoblast, showing two layers separated by fluid-filled spaces. The outer layer of cells (arrowed) is regarded as extra-embryonic mesoderm. Note also the large vacuoles in the giant cell trophoblast. Iron haematoxylin and orange G. $\times 400$.

VARIATIONS IN THE METACARPAL BONES

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Apart from descriptions in standard text-books of Anatomy, few descriptions of the metacarpal bones are available. There are no differences in the accounts of these bones given by Brash (1951), Breathnach (1958), Frazer (1940), Johnston & Whillis (1954), Thane (1893), and Wood Jones (1949), in the respective text-books edited by them. Apart from the reference to occasional absence of one of the facets on the medial side of the base of the third metacarpal (Johnston & Whillis, 1954), or on the lateral side of the base of the fourth metacarpal (Wood Jones, 1949), no variations in these bones have been described. That such a description is required is indicated by the high frequency of variations in these bones.

This paper presents the results of an examination of 100 specimens, 50 right and 50 left, of each of the metacarpal bones. It is not considered necessary to give a detailed description of each bone, as most of the descriptions referred to above are adequate in most respects. This account is, therefore, confined to a description of the variations encountered. There are no significant differences in the findings in bones of the two sides, and hence these are not described separately.

OBSERVATIONS

First metacarpal

The anterior part of the articular surface of the head of this bone is prolonged proximally to form two areas, medial and lateral, over which the sesamoid bones glide. One or both of these facets is missing in 12 out of 100 specimens examined; the medial in five, the lateral in two, and both in five specimens. In the specimens in which both areas are present (88), the lateral is generally the larger (40), but it may equal the medial in size (28), or may even be smaller than it (20).

The anterior aspect of the shaft of this bone is divided, by its anterior border, into a smaller antero-medial and a larger antero-lateral surface. Rarely, however, the two parts are equal in size. In these cases, the side of the body to which the bone belongs may be correctly determined by means of a rough prominence present on the lateral side of its base.

According to Wood Jones (1949), the nutrient foramen to this bone invariably enters the antero-medial surface of the shaft. This is true of 77 specimens examined in the present series. However, five of these show additional foramina; four on the antero-lateral surface, and one on the dorsal surface. In four specimens there is a single foramen piercing the antero-lateral surface, and in one specimen a single foramen is seen on the dorsal surface. Ten bones show a foramen piercing the anterior border. In eight specimens, no distinct nutrient foramen is present.

According to Frazer (1940), and Breathnach (1958), the lateral part of the proximal

articular surface of this bone is larger than the medial. This is true of 55 specimens of the present series. Usually, the extent of this area distally, on the lateral side of the base, exceeds that on the medial, but this finding is not invariable, as will be clear from Table 1.

Table 1. *Proximal articular surface of the first metacarpal*

Relative size of lateral and medial parts			Distal extent on the lateral and medial sides		
Lateral larger	Both equal	Medial larger	Lateral greater	Both equal	Medial greater
78*	17**	5***	55 { 52* 3**	32 { 15* 12** 5***	13 { 11* 2**

The asterisks enable a correlation to be made between the findings in the left and the right halves of the Table.

Second metacarpal

The facet on the medial side of the base of this bone, for articulation with the base of the third metacarpal, is elongated and has a notched distal margin, in 82 specimens (Fig. 1*a*). This conforms to the usual description of the facet. In fourteen specimens, however, it is not notched and is a simple elongated facet (Fig. 1*c*). In four other specimens there are two distinct anterior and posterior facets (Fig. 1*b*). In the 86 specimens showing partial or complete subdivision of the facets into two parts, the anterior part is the larger in 62 specimens, the two parts are of equal size in eight specimens, while the posterior part is larger in the remaining 16 specimens.

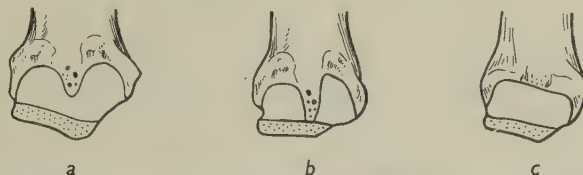


Fig. 1. Medial side of the base of the left second metacarpal bone showing the various forms of the facet for the third metacarpal bone. The stippled area is for the capitate.

The area for the capitate is variable in extent. In 27 specimens it is a well-defined area, extending antero-posteriorly between the facet for the trapezoid and the articular surface for the third metacarpal (Fig. 2*a*). In the majority of specimens (56), however, it tapers towards the posterior margin of the base (Fig. 2*b*). In the remaining 17 specimens it is confined to the region adjoining the anterior part of the facet for the third metacarpal bone (Fig. 2*c*).

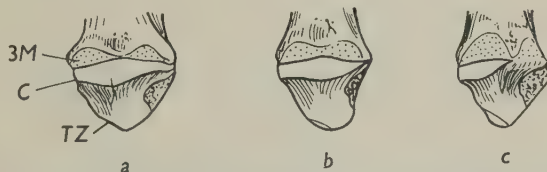


Fig. 2. Infero-medial aspect of the base of the left second metacarpal bone showing the variation in the area for the capitate. 3M, area for third metacarpal; C, area for capitate; TZ, area for trapezoid.

The facet for the trapezium is very constant in occurrence, position and direction. The area for the trapezoid is triangular with its base directed medially, and apex laterally. The facet for the trapezium adjoins the lateral end of the anterior border of this triangle, near the apex. It faces forwards, proximally and laterally, the predominant direction being forwards (Fig. 3).



Fig. 3. Proximal and anterior aspect of the base of the left second metacarpal bone showing the location of the facet for the trapezium. *TM*, area for trapezium; *TZ*, area for trapezoid; *C*, area for capitate.

In 100 specimens of this bone examined by Wood Jones (1949), the nutrient foramen is situated on the antero-medial surface in 59, and on the antero-lateral surface in 41. In the present series the foramen is on the antero-medial surface in 60 specimens, on the antero-lateral surface in 15 specimens, and on the border separating the two surfaces in two specimens. In 19 specimens foramina are present on both surfaces. No nutrient foramen is found in four specimens.

Third metacarpal

The facet on the lateral side of the base of this bone, for the second metacarpal, is elongated and notched distally, in 74 specimens (Fig. 4*a*). There are two distinct facets, anterior and posterior, in 14 specimens, the facets being widely separated in two of them (Fig. 4*b, c*). A single facet without a notch, and very variable in shape is seen in 12 specimens (Fig. 4*d-g*). In 88 specimens showing partial or complete subdivision of the facet into anterior and posterior parts, the anterior



Fig. 4. Lateral side of the base of the left third metacarpal bone showing the various forms of the area for the second metacarpal bone.

is larger in 44 specimens, the two are equal in size in 26 specimens, and the posterior part is larger in 18 specimens.

The medial side of the base of this bone presents two rounded distinct facets in 39 specimens (Fig. 5*a*). The two facets are partially united in 19 specimens, in some cases producing a facet identical with the typical facet seen on the lateral side of the base of this bone (Fig. 5*b-d*). A single elongated oval or rectangular facet, without a notch, is seen in five specimens (Fig. 5*e*). A single rounded facet, confined to the posterior part of the medial side of the base, is seen in 22 specimens (Fig. 5*f*). This obviously represents a condition, wherein the anterior of the two facets seen in the typical specimen is absent. The opposite condition, namely the absence of the posterior facet, is seen in 14 specimens (Fig. 5*g*). Finally, no facet is present in one specimen. In the specimens showing distinct or partially united, anterior and posterior facets (58), the anterior is larger in 22 cases, the posterior is larger in 20 cases, and the two are equal in 16 cases.



Fig. 5. Medial side of the base of the left third metacarpal bone showing the various forms of the area for the fourth metacarpal bone.

According to Wood Jones (1949), the nutrient foramen to this bone is invariably on the antero-lateral surface. This is the case in 90 specimens in the present series. Six specimens show a foramen on the antero-medial surface, two show foramina on both antero-medial and antero-lateral surfaces. In two other specimens no distinct foramina are seen.

This bone is usually identified, and its side determined, by means of its styloid process. In some specimens this process is inconspicuous. In these cases the side to which the bone belongs can be accurately determined by examining the dorsal side of its base, the lateral part of which is distinctly proximal to the medial.

Fourth metacarpal

The lateral side of the base of this bone presents distinct rounded, anterior and posterior facets in 54 specimens (Fig. 6*a*). These facets are partially united in 16 specimens; the union being proximal in 13, and central in three specimens

(Fig. 6*b, c*). The anterior of these facets is absent in 21 specimens (Fig. 6*d*), and the posterior is absent in six specimens (Fig. 6*e*). Finally, three specimens show one elongated facet of variable shape (Fig. 6*f*). In the 70 specimens showing completely or partially distinct facets, the anterior is larger in 30 specimens, and the posterior in 20 specimens; in the remaining 20 specimens they are equal in size.



Fig. 6. Lateral side of the base of the right fourth metacarpal bone showing the various forms of the area for the third metacarpal bone.

The posterior of the two facets on the lateral side of the base of this bone is usually described as being continuous, proximally with a small area for the capitate. This area is present in 62 specimens and absent in 38. It may be pointed out here that the area for the capitate is not in the same plane as the facet for the third metacarpal, but is nearly at right angles to the latter and faces proximally.

The facet on the medial side of the base of this bone, for articulation with the fifth metacarpal, is typically oval and is equally prominent in its anterior and posterior halves, in 70 specimens (Fig. 7*a*). In 25 specimens it tapers anteriorly (Fig. 7*b*). In three specimens it is confined to the anterior part of the medial side of the base of the bone, and in two cases is confined to the posterior part (Fig. 7*c, d*).

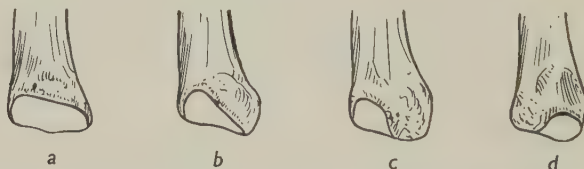


Fig. 7. Medial side of the base of the right fourth metacarpal bone showing the various forms of the area for the fifth metacarpal bone.

The nutrient foramen of this bone is described by Wood Jones (1949) as being always on the antero-lateral surface. In the present series, it is on the antero-lateral surface in 80 specimens, on the antero-medial surface in eight specimens, and on the border between the two surfaces in three specimens. Five specimens show foramina on both surfaces, and no foramina are found in four specimens.

Fifth metacarpal

The facet for the fourth metacarpal often fails to reach as far anteriorly as the proximal articular surface of this bone. Wood Jones (1949) states that the nutrient foramen to this bone is always on the antero-lateral surface. In the present series, the nutrient foramen is on the antero-lateral surface in 86 specimens, on both antero-lateral and antero-medial surfaces in nine specimens, and on the anterior border in two specimens. No foramen is found in three specimens.

From what has been said above it will be clear that the metacarpal bones very frequently show variations from the typical text-book description. The absolute uniformity in the descriptions by the various authors quoted above, leads one to wonder whether the variations encountered are only found in Indian bones. The possibility of these differences having been acquired as a consequence of differences in function cannot be entertained, as there are no apparent differences in the manner in which the hand is used in different races. It is therefore possible that an examination of bones in other races might reveal similar variations.

SUMMARY

1. The variations encountered in 100 specimens of each of the metacarpal bones have been studied.
2. The facets for articulation of the metacarpal bones with one another show very frequent variations from the typical text-book description.
3. The site of entry of the nutrient artery into the bones is also subject to considerable variation.

I am indebted to Dr Indarjit, Professor of Anatomy, Medical College, Amritsar, for permission to examine some material in his department.

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REVIEWS

Gray's Anatomy. Descriptive and Applied. Edited by T. B. JOHNSTON, D. V. DAVIES and F. DAVIES. 32nd edition. (Pp. xiii+1604; 127 illustrations, many in colour; £6. 6s.) London: Longmans Green and Co. 1958.

Normally, owing to exigencies of space, it is new books alone that are reviewed in this *Journal*. The appearance of the centenary edition of *Gray's Anatomy* is, however, surely sufficient justification for breaking this well-established custom. It would be inappropriate, if not even an act of temerity, to attempt to review in the usual manner a volume that has had such a long and successful history and of which thirty-one editions have already appeared. A book which has satisfied so many for so long and which has established itself as a classic presentation of the topography of the human body needs no particular recommendation. Nevertheless, it can be stated that the present editors have carried out their duties with care and skill. The text has been carefully reviewed and many new and attractive illustrations, even including electron micrographs, have been introduced. Furthermore, Prof. Johnston's foreword on the first hundred years of 'Gray', with the associated portraits of previous editors, gives a special interest to this edition.

The editors and the publishers must be congratulated on the centenary of their charge. May its next hundred years be as successful and as useful as its first century has been!

J. D. BOYD

The Respiratory Muscles and the Mechanics of Breathing. By E. J. MORAN CAMPBELL, M.D., Ph.D., B.Sc. (Lond.), M.R.C.P. (Lond.). (Pp. 131+xvi; octavo; 20s.) London: Lloyd-Luke (Medical Books) Ltd. 1958.

In this lively and interesting book of 119 pages of text and 160 references, Dr Moran Campbell is the first to collect the scattered literature upon the subject and review together the muscles engaged in respiration. He considers electromyography, despite its many limitations, the best method hitherto available in the study. The review of the literature is clear, brief and moreover critical, while his own experiments, opinions and inferences make an original contribution enhancing the value of the monograph even if certain points are controversial. There is a very business-like summary to each section and a direct statement if the action of a muscle has not yet been definitely established. An excellent chapter of four pages deals with the nature of the limitation of maximum inspiratory and expiratory efforts. An appendix discusses the technique of electromyography, the apparatus used and its management and experimental procedure. The following quotation from the introduction will give the potential reader the best idea of the author's method and style:

'In the particular case of a respiratory muscle, descriptions of its mechanical action and of its contractile activity must be supplemented by a final assessment of its respiratory function. These three stages in the elucidation of its properties must be distinguished from each other. For example, the electromyographic finding of activity in an intercostal muscle during inspiration does not prove that its mechanical action is to elevate the lower rib to which it is attached; nor does it prove that its function is to aid inspiration by enlarging the thorax. The elevation of the lower ribs may have been produced by other muscles, and the contraction of the intercostal muscles may only have served the function of maintaining the tension of the intercostal spaces. The importance of these distinctions has been stressed because failure to observe them has been a major source of confusion in the literature of the subject.

Thus for a full description of the function of a muscle of breathing we may require the

following data: its mechanical action; the circumstances of its contraction in relation to the phases of respiration and in various respiratory manoeuvres; and an evaluation of its respiratory function in terms, say, of intrapleural pressure changes produced by its contraction.

The extent to which these requirements can be met is variable. There are surprising uncertainties in our knowledge of the mechanical action of several of the important muscles. The circumstances of contraction of some of them are difficult to determine. The measurement of the force contributed by individual muscles to the mechanics of breathing has rarely been attempted.'

So few bouquets come the way of the classical anatomist that one cannot refrain from citing the last sentence of the author's preface quoting Dr W. O. Fenn: 'The mechanics of breathing is a problem requiring on one hand the detailed knowledge of a classical anatomist and on the other hand the analytic understanding of an engineer.'

R. D. LOCKHART

Peripheral Nerve Injuries. By RUTH E. M. BOWDEN. (Pp. viii + 60; 29 illustrations; 1 table; 8s. 6d.) London: H. K. Lewis and Co. Ltd. 1958.

This is an authoritative, clearly written, much needed book; moreover, it is commendably short. Medical students, in particular, should be encouraged to read it, for it demonstrates the functional significance of the structure they see when they are dissecting the limbs of preserved bodies.

G. WEDDELL

Methods for Research in Human Growth. By S. M. GARN and Z. SHAMIR. (Pp. 121; 36s.) Springfield, Illinois, U.S.A.: Charles C. Thomas. 1958.

Amongst the half-dozen or so major longitudinal growth studies of children the Fels Research Institute for the Study of Human Development may justly claim pride of place. Begun nineteen years ago in quite a modest way it has been steadily and sensibly developed by its Director, Dr Lester Sontag, until now it has the status of a major Research Institute with a score of workers in anthropology, genetics, biochemistry, physiological psychology and other disciplines. Though its central core remains the longitudinal study of a limited number of children throughout their growing period, many cross-sectional problems, not all of them necessarily directly a part of child development in the narrow sense, are tackled, and monkeys and mice frequent the building as well as anthropologists and children.

Dr Sontag has been lucky, or perhaps wise, in his choice of physical anthropology staff. For many years this department was under the care of Dr Earl Reynolds, well known for his studies of the development of the pelvis in childhood, the genetics of ossification patterns, and the differential growth of bone, muscle and fat in the limbs, and, later and to a different audience, for his accounts of his ketch's progress round the more delectable oceans and islands of the world. After him came Dr Stanley Garn, another ready wielder of pen as well as pinch-calipers.

Dr Garn's present book, written in collaboration with Dr Zvi Shamir of the Department of Pediatrics at the Jerusalem Medical School, is short, very well written, and throughout informed by a deal of common sense, great practical experience of growth study work, and a refreshing sense of humour. As the authors say, 'it is intentionally modest. It is more of a guide to methods than a handbook of growth research. Any of the chapters could be expanded to book length.' There are chapters on planning a growth study, the pediatric examination, the taking of anthropometric and roentgenometric measurements, the way the development of teeth, skin, hair, muscle and fat, sexual maturation and strength may be followed. In practically none of these are actual techniques of measuring discussed, nor critical evaluation of competing techniques attempted. The book is a signpost simply to the sort of things people in growth studies do. Some physiological and biochemical procedures are signposted also, but not psychological ones. There is a chapter on the statistical

analysis of growth data again of a sign-posting rather than critical nature, and a final chapter on graphical representation of growth data, which in the reviewer's opinion is the best chapter in the book.

There are of course a number of points where the reviewer's judgement is at odds with the writers': in their recommendation of the Kodlin-Thompson monograph an appraisal of longitudinal data as 'authoritative'; in the rather cavalier dismissal of the Oxford points system of assessing bone age; in the belief that seasonal differences in weight gain are only characteristic of clothed and not nude subjects; in details of the technique of measuring muscle by X-rays; and, when discussing the measuring of subcutaneous fat by the use of skinfold calipers, in the failure to quote the paper of Edwards and his colleagues (*Brit. J. Nutr.* 9, 133, 1955) which constitutes by far the best-controlled and most thorough test of any anthropometric instrument yet carried out.

These are, however, the inevitable cavillings of brother technicians. Garn and Shamir's book is a very pleasant evening's reading—it takes just about 1½ hours—and thoroughly to be recommended to anatomists, anthropologists, pediatricians and all others who wish to get an idea what sort of things their colleagues who study growth are up to.

J. M. TANNER

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DEVELOPMENT AND POSTNATAL CHANGES OF DIGITAL PACINIAN CORPUSCLES (*CORPUSCULA LAMELLOSA*) IN THE HUMAN HAND*

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LITERATURE

Lamellar corpuscles were first observed in the digital tissues more than 200 years ago (Vater, 1741), but their existence was forgotten until a hundred years later, when they were rediscovered by Pacini (1835, 1840). Since then, the structure, function and distribution of the corpuscles have attracted considerable attention; their development, however, has been studied by very few anatomists. Virchow (1858) suggested that the corpuscles represented 'colossal development of perineural elements around a single nerve fibre' and considered that they might be abnormal formations. Key & Retzius (1876) also considered that the corpuscles were derived from axonal sheaths but as a normal biological development, but Ranvier (1875) found that the lamellar cells were endothelial in nature. Krause (1881) suggested that all terminal corpuscles had the same general organization and were derived from the sheaths of the nerve fibres, and he introduced the terms inner and outer bulbs (Innenkolben, Ausserkolben): the inner bulb consisted of cells derived from the Schwann sheath, and the outer bulb or capsule was fibrous and developed from the perineural tissues.

Systematic study of the development of the Pacinian corpuscles was first undertaken by Tello (1922) on chick embryos, using the Cajal's block silver method. He found that the corpuscles were first formed near the interosseous membrane of the leg in 13 to 14-day-old chicks. After 15 days, the periphery of the corpuscles showed a capsule, while the centre still remained a mass of embryonic connective tissue.

The development of human digital corpuscles was systematically studied by Pilate (1925) by the use of various nerve staining methods and counterstaining. He found that the development of the corpuscles began in foetuses of 7 cm. body length 'as accumulation of cells around the end of a growing nerve fibre which was devoid of a sheath at that stage'. The nerve fibre produced short fine branches all of which ended in club-shaped condensations. In foetuses of 8-8.5 cm. of body lengths, a sheath appeared around the corpuscles followed by new layers. In foetuses of 13-14 cm. body lengths, five or more lamellae could be counted, and the ramification of the central axon became more extensive. In foetuses of 16.5, 17, 21 and 22 cm. lengths, the corpuscles appeared similar in structure to the definitive forms. Levi (1933), studying the development of the digital nerves by Cajal-De Castro

* Part of this work was submitted in partial fulfilment of the requirement for the degree of Ph.D. in the University of Durham by one of us (G. M.)

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method, found that Pacinian corpuscles first appeared during the fourth foetal month and that their development followed the same pattern as in the chick described earlier by Tello; but Levi suggested, in addition, that the corpuscles increased in number by longitudinal cleavage. Lambertini (1956), in his studies of the development of the digital nerves and terminal receptors in foetal fingers by the Ruffini's gold chloride method, found that Pacinian corpuscles developed much earlier than the receptors of the papillary layer.

The development of human retro-peritoneal Pacinian corpuscles was investigated by Takashi (1957) by the use of Ranson's silver impregnation method and Morgan's stain for myelin sheaths. He found that the development commenced in foetuses of 14-16 cm. body length as accumulation of cells around the end of a nerve fibre which sometimes showed ramification; this primordium of the corpuscle was incompletely encapsulated by the surrounding fibroblasts. Earliest lamellar formation and differentiation of the inner bulb around the branching axon started in foetuses of 25 cm. body length. In foetuses of 28 cm. length, capillaries were observed around the corpuscle and within the outer bulb. Inter-lamellar spaces made their appearance at about 45 cm. stage (9th foetal month), and the whole structure became mature one and a half years after birth. He thought that the inner bulb was derived from Schwann cells, and the lamellae from fibroblasts of the endoneural sheath rather than from the surrounding connective tissue.

Postnatal lamellar corpuscles have been investigated in various situations in man and in vertebrates. They have been described in the digital tissues including the joints, periosteum, interosseous membranes and muscles (Henle & Kölliker, 1844; Herbst, 1848; Rauber, 1865, 1867; Hartenstein, 1889; Leontowitsch, 1901; Van de Velde, 1909; Aiba, 1956; Cauna & Mannan, 1958; Miller, Ralston & Kasahara, 1958). Pacinian corpuscles have been found in the adventitia of large blood vessels (Arndt, 1875; Thoma, 1884; Woollard & Weddell, 1935; Bertelli, 1953; Lodone, 1953; Comparini, 1954), in the glomus coccygeum region (Schumacher, 1908, 1911), mesentery and peritoneum (Key & Retzius, 1876; Dogiel, 1902, 1905; Ramström, 1908; Takashi, Saki & Usizima, 1955).

Lamellar corpuscles have also been described in various domestic and wild animals, especially in the mesentery and mesocolon of the cat (Jacubowitch, 1860; Krause, 1863; Hoyer, 1864; Schäfer, 1875; Sala, 1899; Sokolow, 1899; Michailow, 1908; Dogiel, 1910; Quilliam & Sato, 1955; Pease & Quilliam, 1957), in external and internal genitalia of various animals (Timofeew, 1896; Shimizu, 1954), limbs of the bat (Grosser, 1901), and in domestic and wild birds (Leydig, 1954, 1868; Van de Velde, 1909; Botezat, 1912; Tello, 1922; Clara, 1925).

Postnatal change of Pacinian corpuscles have not been systematically investigated as far as can be gathered from the available literature, but some valuable information for such a study is contributed by Winkelmann & Osment (1956), who investigated the external form of the digital Pacinian corpuscles of an adult individual by the wax plate reconstruction method. A similar study was carried out by Takashi, Saki & Usizima (1955) on human retro-peritoneal corpuscles. These observations show that the adult structures occur in a large variety of shapes and sizes which are apparently derived from the simple bulbous foetal predecessors.

The purpose of this study is to follow the life history of the digital Pacinian

corpuscles, from the earliest stages of their development to the complex forms of postnatal life, in order to understand the mechanism and causes of their transformation and the place of the corpuscle among other receptor organs.

MATERIAL AND METHODS

Human material used in this work consists of hands of fifty-one foetuses between 55 mm. crown-rump length and full term, and fingers of forty-nine infants and children from birth to 10 years of age. In addition, postnatal changes of the corpuscles were studied in adult individuals of all age groups. Visceral lamellar corpuscles were examined in the mesentery and mesocolon of eight cats aged from 1 week to adult life. The distribution of human prenatal material is shown in Table 1.

Table 1. *Distribution of foetal material*

c.r. length (mm.)	No. of cases
55-60	3
61-70	2
71-80	2
81-90	4
91-100	5
101-120	9
121-150	14
151-180	6
181 to full term	6
Total	51

Most specimens were fixed in 10% commercial formalin for at least 1 week. Some postnatal biopsy specimens were treated with hyalase for 20 min. (Weddell & Pallie, 1954) before being fixed in formalin. Bouin's solution was used for decalcification of foetal material of up to 100 mm. crown-rump length. Later stages and postnatal specimens were decalcified in Ebner's fluid and neutralized in 5% potassium alum.

Serial paraffin sections were cut at 5-12 μ in thickness orientated parallel and perpendicular to the long axis of the fingers. Frozen sections were usually cut at a thickness of 20 μ in planes parallel and perpendicular to the skin surface.

For general histological study, the following stains were used: Harris's haematoxylin and eosin, Weigert's iron haematoxylin, Van Gieson's picro-fuchsin and polychrome methylene blue. Nerves were stained in frozen and paraffin sections by the simplified Bielschowsky-Gross silver impregnation method as follows: before staining, sections were transferred into distilled water for about 10 min.; then they were put in 20% silver nitrate solution for 10 min.; three changes of 3% commercial formalin prepared with tap water; ammoniacal silver nitrate solution, 5 min.; two changes of 3% formalin, 5 min.; toning in 0.05% gold chloride solution for several minutes; stained sections were fixed in 5% sodium thiosulphate for approximately 10 min. before being cleared and mounted in DPX. In some cases, the silver sections were counterstained with Harris's haematoxylin or Van Gieson.

Myelin sheaths of nerves in paraffin embedded sections were stained by the Kultschitzky-Pal method, and in frozen sections by Anderson's modification of the above method (Anderson, 1929). In addition, myelin sheaths were studied by

optical polarization in unstained frozen sections mounted in water mounting medium.

The distribution of the Pacinian corpuscles in the radial half of the index finger of a foetus of 204 mm. crown-rump length was found by means of a combined graphic and wax plate reconstruction, and it is based on 223 serial longitudinal sections of the finger.

The external form of Pacinian corpuscles was studied by means of wax-plate reconstructions from serial paraffin sections. The total number of corpuscles reconstructed was thirty-six, comprising thirteen individuals from late foetal stages to 82 years of age.

OBSERVATIONS

The development of the Pacinian corpuscles begins at the stage when the fine branches of the digital nerve have already reached the subepidermal zone, but the corial plexus and the superficial receptors of the skin have not started to evolve. Pacinian corpuscles are usually supplied by whole undivided axons (Pl. 2, figs. 14, 15; Pl. 3, fig. 20; Pl. 4, fig. 24); only as an exception two adjacent corpuscles may share a common fibre (Pl. 4, fig. 25), but they never share nerve supply with other tissues. In this way they possess private lines of communication to the spinal cord, whereas the superficial receptors supplied through the agency of the corial plexus possess heavily shared party lines (Cauna, 1956; Cauna & Mannan, 1958).

In human digital tissues, developing Pacinian corpuscles were first recognized in foetuses of 69.5 and 71 mm. crown-rump lengths as clusters of cells along the branches of the digital nerve in situations where subcutaneous and periosteal groups are later found. At this stage sheath cells have already made their appearance in the nerve. A developing corpuscle can be seen in close relationship with the dorsal branch of the collateral digital nerve in Pl. 1, fig. 1 (arrow). The same corpuscle is shown at a higher magnification in Pl. 1, fig. 2.

The structure of the receptor in its initial stage of development can be seen in three consecutive transverse sections in Pl. 1, figs. 4-6 (arrows). Fig. 4 shows the proximal end of the corpuscle, fig. 5 the middle part, and fig. 6 the free distal end of it; the corpuscle is accompanied by a nerve bundle (*N*) from which it is supplied. Figs. 3 and 7 are two sections of the same series: fig. 3 shows the nerve bundle immediately proximal to the corpuscle, and fig. 7 immediately distal to it. The nerve bundle of fig. 3 after giving a fibre to the corpuscle becomes appreciably smaller (figs. 5-7); it can be seen that the cell nuclei of the developing corpuscle are of uniform character in all parts, and that they show a marked degree of similarity with those of the sheath cells of the nerve. The nerve fibre supplying the corpuscle ramifies intensely in a peculiar 'stag-horn' manner among the cells, and each branch usually ends in a slight condensation. The complex type of ramification can be seen in Pl. 2, fig. 14: the corpuscle is avascular at this stage, but the nearby blood vessel (*V* in fig. 14) will probably be incorporated within the receptor during later development (cf. Pl. 2, fig. 14, with Pl. 3, fig. 20).

At the initial stage, the nerve ending together with the cluster of cells constitute the entire corpuscle: the surrounding connective tissue elements do not show any specific arrangement in relation to it except that some cells adjacent to the receptor may be slightly flattened. In later stages of development, however, there is a rapid

increase in number of these flattened cells which arrange themselves in concentric layers forming the so-called outer bulb or lamellae of the corpuscle (Pl. 2, fig. 17; Pl. 3, fig. 18); while the initial cluster of cells surrounding the nerve fibre then becomes the inner bulb or central core.

Pl. 1, figs. 8 and 9 show the early formation of the outer bulb in a foetus of 81 mm. crown-rump length. Fig. 9 shows two terminal branches of the collateral digital nerve each carrying a developing corpuscle (arrows). Fig. 8 shows a corpuscle reproduced at a higher magnification: it can be seen that the cluster of cells in the centre (arrows) corresponds with the entire corpuscle of the earlier stage (Pl. 1, figs. 2, 5) and the flattened cells surrounding the central core in a concentric manner are a new feature and mark the beginning of the lamellar formation of the outer bulb. Pl. 2, figs. 10–13 show a slightly later stage of development in four consecutive transverse sections from a foetus of 88.8 mm. crown-rump length. In all the four sections, the outer bulb can be recognized as consisting of concentric cellular layers which gradually blend with the surrounding adventitial tissue. The few cells in the centre of the corpuscle in fig. 10 (arrows) constitute the distal end of the central core which can be traced in figs. 11 and 12 as compact cellular formations (arrows). Fig. 13 shows the proximal end of the corpuscle; the tapering central core (arrows) is going to blend with the sheath cells of the nerve supplying the corpuscle.

The outer bulb, when fully formed, consists of thin concentric layers of cellular lamellae with spaces between them which contain some fluid and collagen fibres arranged in a circular manner, as well as blood vessels (Cauna & Mannan, 1958). In early stages the outer bulb is cellular, and the most peripheral cells gradually blend with the surrounding adventitia (Pl. 2, fig. 17). There are no fibres between the layers of these cells and no definite inter-lamellar spaces can be observed. The cellular nature of the outer bulb is demonstrated in Pl. 3, fig. 19, which is taken from a foetus of 125 mm. crown-rump length. The section has been stained with Van Gieson's picro-fuchsin in order to differentiate cytoplasmic material from extra-cellular elements of the tissues. By this method, the cytoplasm stains yellow (light grey in the photograph) and the extracellular fibrous material red (black in the photograph). It can be seen that the Pacinian corpuscle (arrows in fig. 19) is cellular at this stage and is of the same appearance as sweat glands (*S*) or the living cells of the epidermis (*E*). Fibrous tissue, such as the flexor tendon (*T*) and the fibrous framework of the corium, are black, as is the cornified surface layer of the epidermis consisting of dead cells. First appearance of definite fibres in the outer bulb of the Pacinian corpuscle was observed at about 130 mm. stage.

While the development of the lamellae is in progress, significant changes are observed in the nerve ending. The short irregular branches typical of the initial stage gradually disappear and a solitary central axon now occupies the inner bulb (cf. Pl. 2, fig. 14, with Pl. 2, fig. 15, and Pl. 4, figs. 24, 25).

As the corpuscle increases in size, link is gradually established between it and the adjacent blood vessels, the latter becoming incorporated into the lamellar system. Pl. 3, fig. 20 shows a major branch of the collateral digital nerve together with a blood vessel (*V*) from a foetus of 144 mm. crown-rump length, and a branch of the blood vessel can be seen in the process of being incorporated into the developing Pacinian corpuscle (*P*).

The inner and outer bulbs show different development from the very beginning, and the difference increases as the foetus grows older. Pl. 3, fig. 18 shows a transverse section of a Pacinian corpuscle from a foetus of 145 mm. crown-rump length. The specimen has suffered some shrinkage and shows that the inner and outer bulbs can be separated from one another by cleavage. The inner bulb is cellular as at the beginning of its development; it does not show much increase in diameter from the time of the initial stage till its full maturity after birth, but it grows in length throughout the pre- and postnatal life, retaining its cellular character. The outer bulb though still compact at the 145 mm. stage (Pl. 3, fig. 18) shows a few collagen fibres between its cellular layers when stained with Van Gieson's stain; it increases in diameter throughout foetal development and postnatal life by addition of new lamellae.

The central nerve fibre of the Pacinian corpuscle having lost the primary branches of the initial stage of development, lies in an axial position in the inner bulb and is conspicuous by its large diameter when compared with the axons of the adjacent nerve fascicles (Pl. 2, fig. 15; Pl. 3, fig. 20; Pl. 4, fig. 24). In some corpuscles this simple manner of nerve ending may persist throughout the prenatal period of development and may be found postnatally. But in foetuses from 145 mm. crown-rump length onward, the axon may give rise to a second set of branches which end within the distal part of the inner bulb in small enlargements. Usually all branches are of equal or nearly equal sizes, but occasionally the thick axon gives rise to some very fine fibres which in turn ramify and pursue a winding course, often surrounding the principal fibre (Cauna & Mannan, 1958).

By the time the foetus attains the size of 200 mm. crown-rump length, the histo-differentiation of the corpuscle is complete. The cellular lamellae and the inter-lamellar spaces are fully formed and considerable amounts of fibres are present in these spaces. Blood vessels of large capillary type can be found in the outer bulb of all corpuscles when serial sections are examined, and veins leading from them can be traced to the developing arteriovenous anastomoses as found in postnatal life (Cauna & Mannan, 1958). Pl. 3, fig. 22 shows two subcutaneous Pacinian corpuscles cut transversely together with a developing glomerular arteriovenous anastomosis (*AV*) from a foetus of 204 mm. crown-rump length. Lamellae and inter-lamellar spaces are well formed in the corpuscles, and collagen fibres can be easily demonstrated in the inter-lamellar spaces with Van Gieson's stain. A vein (arrows in Pl. 3, fig. 22) draining one of the two corpuscles can be seen to join the venous end of the developing arteriovenous anastomosis (plane of the section is indicated in fig. 23, which shows the two corpuscles and the blood vessels in a drawing from a wax-plate reconstruction).

In foetuses over 204 mm. crown-rump length, fully formed Pacinian corpuscles are found in all layers of the digital tissues (Text-fig. 1); the changes that occur in subsequent development are a progressive increase in size of the corpuscle and gradual ramification of the central nerve fibre. Pl. 3, fig. 21 shows a portion of a corial corpuscle with the central nerve fibre ending in a swelling; it also shows a bud at the side of the stem fibre (arrow) giving rise to a new branch.

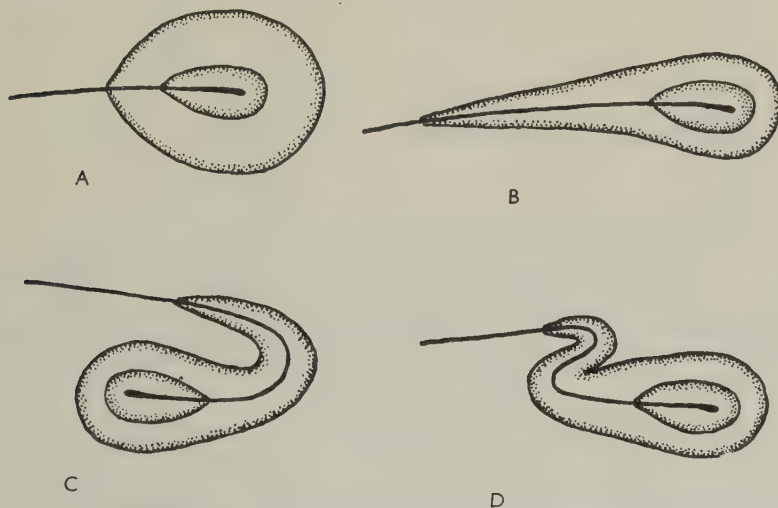
The growth of a Pacinian corpuscle is controlled by two factors: (*a*) apposition of new lamellae over the surface, and (*b*) retrograde growth of the corpuscle along

the nerve fibre (Cauna & Mannan, 1958). As a result, the shape of the corpuscle changes according to which process of growth is dominant at any particular period.

If retrograde and appositional growth rates are equal, the originally elongated corpuscle gradually attains a nearly spherical shape (A in Text-fig. 2); if retrograde growth is rapid, the corpuscle becomes more elongated (B in Text-fig. 2). In order to maintain a constant oval shape of a growing corpuscle, a ratio of approximately



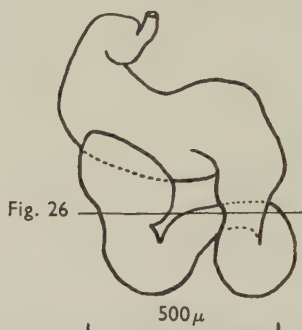
Text-fig. 1. Drawing from a combined wax plate and graphic reconstruction showing the distribution of the Pacinian corpuscles in the radial half of the index finger. Foetus of 204 mm. C.R. length (223 sections, 10μ thickness), $\times 75$.



Text-fig. 2. Diagram showing changes in the external shape of foetal Pacinian corpuscles (in the centre of each diagram) as a result of variations in appositional and retrograde growth rates. A, Appositional and retrograde growth rates are equal—the oval corpuscle gradually attains a nearly spherical shape; B, extensive retrograde growth results in elongation of the corpuscle; C, D, the external shape of the corpuscle is determined by the course of the nerve fibre which is being incorporated into the receptor by retrograde growth.

1:3 is required between appositional and retrograde growth rates. Before entering the corpuscles, nerve fibres usually show some irregularities in their course, and when these are incorporated into the receptors their shapes change accordingly (C and D in Text-fig. 2, and Pl. 3, fig. 23). In certain instances the appositional growth is negligible and the corpuscle grows along the winding nerve fibre into a coiled irregular structure (Text-fig. 3), which may appear as a group of corpuscles examined in histological sections without the use of reconstructions (Pl. 4, fig. 26).

Coiled corpuscles, such as shown in Text-fig. 3, may become more complex in structure when adjacent coils become linked together by apposition of a common lamellar system around them; in histological sections such corpuscles (Pl. 4, fig. 27) may appear as having two or more inner bulbs supplied by independent nerve fibres.



Text-fig. 3. Drawing from a wax plate reconstruction of a subcutaneous Pacinian corpuscle showing a remarkable manifestation of retrograde growth. Distal phalanx, index finger. Male, 22 years (64 sections, 12μ thickness). $\times 50$.

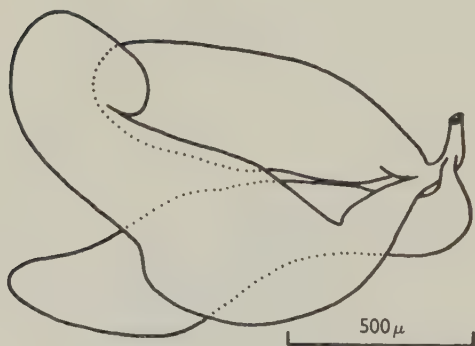


Fig. 4



Fig. 5

Text-figs. 4, 5. Drawings from wax-plate reconstructions showing gradual fusion of independent corpuscles as a result of retrograde growth. Text-fig 4. Distal phalanx, middle finger. Female 30 years (72 sections, 12μ thickness). $\times 50$. Text-fig. 5. Distal phalanx, index finger. Male, 6 years (82 sections, 10μ thickness). $\times 50$.

Extensive retrograde growth along the nerve fibre in combination with subsequent apposition of common surface lamellae may result in partial or complete fusion of originally independent corpuscles. This can be seen in Text-figs. 4 and 5. Text-fig. 4 shows three corpuscles about to fuse; Text-fig. 5 shows two corpuscles which have already fused at their proximal ends. Further fusion gives origin to composite corpuscles with several inner bulbs each actually supplied by an independent nerve fibre.



Fig. 6

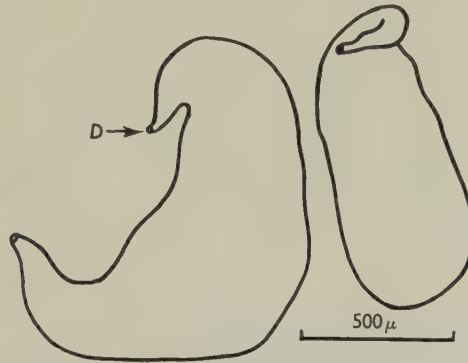


Fig. 7

Text-fig. 6. Drawing from a wax-plate reconstruction showing a segmented Pacinian corpuscle. Distal phalanx, index finger. Male, 6 years (172 sections, 10μ thickness). $\times 50$.

Text-fig. 7. Drawing from a wax-plate reconstruction showing a corpuscle of the usual appearance (on the right) and a 'spindle-shaped corpuscle' (on the left). *D*, distal extremity containing an extension of the inner bulb devoid of the nerve ending. Distal phalanx, little finger. Male, 17 years (108 sections, 10μ thickness). $\times 50$.

In long corpuscles, the appositional growth usually proceeds at different rates in different parts of the same corpuscle, resulting in a sequence of enlargements separated by constrictions (Text-fig. 3), which sometimes consist of few lamellae only; as a result, segments are formed each of which appear as an independent corpuscle. Such cases can best be observed in the mesentery which can be studied in whole mounts (Pl. 4, fig. 28). In the digital tissues, plate reconstructions are required to demonstrate 'segmented corpuscles' (Text-fig. 6).

Some corpuscles in the digital tissues differ from the various forms already

described, both by their external form and internal organization: they are spindle-shaped at both ends and the proximal extremity can only be recognized by the nerve fibre entering it. The inner bulb traverses the whole length of the corpuscle, but its distal extension is devoid of a nerve ending and resembles the 'ligamentum intercapsulare' of Pacini (1840). A spindle-shaped corpuscle is shown in Text-fig. 7 (on the left) along with one of the usual appearance (on the right).

In foetal corpuscles and in those of infants the myelin sheath ends as the nerve fibre enters the receptor (*M* in Pl. 4, fig. 29), but later in life, the sheath can be traced inside the corpuscle for a very considerable distance as a result of retrograde growth of the lamellae (Cauna & Mannan, 1958). In such cases transition of the medullated axon into the nerve ending is gradual, and the cells of the Schwann sheath appear to change into those of the inner bulb after myelin has disappeared. In this way the inner bulb grows on account of the medullated axon, but this process is slower than the general longitudinal growth of the corpuscle.

In segmented corpuscles, such as shown in Text-fig. 6 and Pl. 4, fig. 29, the myelin sheath usually traverses the whole of the proximal segment and the intermediate constriction to end within the distal segment.

Postnatal growth of the Pacinian corpuscle does not proceed evenly and continuously as observed in Meissner's touch corpuscles (Cauna, 1956, 1958). Some of them change little, while others undergo significant transformation with the result that in advanced age they vary considerably in size and shape even in the same individual.

DISCUSSION AND CONCLUSIONS

The development of the digital Pacinian corpuscles begins earlier than that of the corial plexus and the superficial receptors, and can be subdivided into three distinct periods or stages: (1) initial or primordial, (2) avascular, and (3) vascular.

The initial development begins in foetuses of 70–90 mm. crown-rump lengths according to deep or superficial situations of the receptors and extends to approximately 120 mm. stage. During this phase the corpuscle consists of a nerve ending which ramifies in a peculiar 'stag-horn' manner, surrounded by a cluster of cells, all of which appear to be of one type and correspond with those which later constitute the central core or the inner bulb (Pl. 2, figs. 14, 16). The cells are similar in appearance and continuous with those forming the Schwann sheath of the supplying nerve (Pl. 1, figs. 3–7). This finding indicates that the inner bulb is derived from lemmoblasts as originally suggested by Krause (1881). Supporting the evidence is the observation that in postnatal corpuscles the Schwann sheath gradually blends with the cells of the central core.

The second or avascular stage is characterized by the development of the lamellae or the so-called outer bulb around the periphery of the primordial corpuscle and by reduction and loss of the branches of the nerve ending (Pl. 2, figs. 15, 17).

The newly formed lamellae are clearly demarcated from the cells of the central core, but at the same time they blend with the surrounding adventitia. This finding strongly supports the suggestion made by Virchow (1858) that the outer bulb is of adventitial or perineural origin.

The third or vascular stage can be associated with maturation of the lamellae and growth of the corpuscle whereby some of the surrounding blood vessels are gradually

incorporated into the outer bulb (Pl. 3, fig. 20). Later, during this stage the vascular segment draining the Pacinian blood vessels develops into a glomerular arteriovenous anastomosis (Pl. 3, figs. 22, 23), whereby the final organization of the receptor is completed except for its growth in size and eventual secondary ramification of its nerve fibre within the central core.

After birth, growth of Pacinian corpuscles continues both in diameter by apposition of new lamellae and in length by retrograde growth whereby the medullated axon is progressively incorporated into the receptor.

The majority of corpuscles increase in size harmoniously undergoing little change in shape, but some of them are transformed considerably by extensive retrograde growth followed by an increase in diameter of the newly added extension (Text-figs. 3, 6). A question arises as to the significance of the transformation of some corpuscles. An answer to this can only be offered in the form of a suggestion which requires further evidence. Digital Pacinian corpuscles have a complex relationship with the glomerular arteriovenous anastomoses designed to signal changes in local blood supply (Cauna & Mannan, 1958), and this relationship may be disturbed as a result of changes in the surrounding tissues. Since blood vessels are always found in company with nerves, the developmental process similar to that of the second and third stages can be repeated proximal to the existing corpuscle on the same nerve fibre, and when adequate relationship with the blood vessels is established the newly formed extension may replace the original receptor which thus may undergo involution. In Text-fig. 3 the enlarged proximal segment may actually represent the reconditioned part of the receptor while the winding distal portion may be undergoing reduction. In case of the segmented corpuscles (Text-fig. 6; Pl. 4, fig. 28), the proximal segment may be replacing the older distal one which later may disappear altogether. This would explain the occurrence of the spindle-shaped forms with the central core reaching the distal surface (Text-fig. 7, corpuscle on the left, cf. with Text-fig. 6).

Terminal nervous corpuscles are usually considered as static structures when their development is completed, but according to the theory just outlined Pacinian corpuscles possess dynamic qualities throughout life and are adaptable to environmental changes. Recent observations that nerve endings in epithelia and in certain corpuscles undergo a continuous process of growth and involution are in support of this theory (Cauna, 1959).

During foetal development, the digital Pacinian corpuscle shows major progressive and retrogressive changes. Progressive ones are the acquisition of the lamellar outer bulb and the establishment of a link with the arteriovenous anastomoses; a retrogressive change is signified by the loss of branches of the nerve ending after the initial ramification. Major changes in a developing organ frequently reflect stages of evolution, and it may be useful to analyse the development in such terms especially since these corpuscles have a wide distribution among mammals and birds.

It is well known that the lamellar corpuscles occur in a variety of forms according to their situations and the species of vertebrates. Mesenteric corpuscles in mammals usually contain a single central nerve ending (Gray & Malcolm, 1950; Quilliam & Sato, 1955) and the outer bulb appears to be avascular in most cases (Sheehan, 1933*b*); but a network of fine blood vessels usually surrounds the surface of the

outer bulb in the form of a basket (Pl. 4, fig. 30). In a similar way, small Pacinian corpuscles of birds, also known as Herbst's corpuscles, have simple nerve endings and are devoid of blood vessels, but they are frequently embedded in cavernous tissue (Leydig, 1868). By structure, the definitive forms of mesenteric and avian lamellar corpuscles closely resemble the second developmental stage of the digital Pacinian corpuscles, and they may actually represent an arrested stage in evolution which happens to be adequate for the simpler functional requirements of the regions they supply.

In addition to the 'typical forms' the mesentery and other viscera of mammals contain a variety of 'simple lamellar corpuscles' (Sheehan, 1933*a*; Shimizu, 1954). Similar simple forms occur in other deep tissues, especially in association with aponeuroses, tendons and joints (Pansini, 1955; Stilwell, 1957*a-c*). The histological description of such receptors, like their terminology, is usually vague, and their development has not yet been sufficiently investigated, but the available information may justify a tentative suggestion that these simple end organs are modifications of one original form derived from the primordial Pacinian corpuscle which may signify an atavistic receptor of the deep tissues.

SUMMARY

1. Development and postnatal changes of the digital Pacinian corpuscles were studied in hands of fifty-one foetuses and in fingers of forty-nine infants and children, as well as in adults of various ages, by the use of cytological and nerve staining methods, and wax-plate reconstructions.

2. It was found that the Pacinian corpuscles started their development earlier than the superficial receptors and that they evolved in three stages.

3. During the initial or primordial stage, the corpuscle consists of a nerve ending which branches in a 'stag-horn' manner, surrounded by a cluster of cells of lemmoblastic origin which later becomes the inner bulb.

4. During the avascular stage, the nerve fibre loses its branches and cellular lamellae develop from the adventitial tissue giving origin to the outer bulb.

5. During the vascular stage, spaces and fibres appear between the lamellae and blood vessels related to the surface of the corpuscle are incorporated into the outer bulb by apposition of new lamellae; the central nerve ending may undergo a secondary ramification.

6. After birth, the corpuscles increase in diameter by apposition of lamellae and in length by retrograde growth whereby the medullated axon is progressively incorporated into the receptor.

7. Lamellar corpuscles with complex inner bulbs are derived either from long winding corpuscles when the coils become linked together by a common lamellar system, or from adjacent independent corpuscles by fusion.

8. When vascular relationship between a corpuscle and an arteriovenous anastomosis is disturbed, a new receptor is formed on the same axon by retrograde growth of the existing corpuscle which subsequently undergoes involution.

9. It is suggested that the development of the digital Pacinian corpuscle reflects the evolution of the deep receptors and that the primordial stage of the corpuscle signifies an atavistic receptor from which the deep end organs have evolved by specialization.

We should like to thank Mr C. J. Duncan and the staff of the Photography department for their aid with the photographic work, and Miss Dorothy Mustart for preparing the drawing of Text-fig. 1.

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EXPLANATION OF PLATES

All illustrations, with the exception of figs. 28 and 30, are photomicrographs of human digital skin or subcutis taken from the palmar aspect of fingers and thumbs. Figs. 28 and 30 are photomicrographs of mesenteric Pacinian corpuscles of the cat. An 0.8 mm. oil-immersion objective was used for figures reproduced at a magnification of $\times 1200$.

PLATE 1

- Fig. 1. Longitudinal section through the head (H) and the base (B) of the proximal phalanx showing a developing Pacinian corpuscle (arrow) in relation to the dorsal branch of the collateral digital nerve. Index finger. 71 mm. c.r. length. H. & E., 8μ . $\times 60$.
- Fig. 2. Higher magnification of the developing Pacinian corpuscle (arrow) from fig. 1. N, nerve bundle from which the corpuscle is supplied. Proximal phalanx, index finger. 71 mm. c.r. length. H. & E., 8μ . $\times 1200$.
- Figs. 3-7. Three consecutive serial transverse sections of a developing Pacinian corpuscle (arrows), together with a bundle of nerve fibres (N) from which the corpuscle is supplied. Fig. 4, proximal, fig. 5, middle, and fig. 6, distal, parts of the corpuscle. Figs. 3 and 7, sections of the nerve bundle immediately before and after the sections of the corpuscle. Distal pad, middle finger. 71 mm. c.r. length. H. & E., 8μ . $\times 1200$.
- Fig. 8. Transverse section of an early developing Pacinian corpuscle with the inner bulb (arrows) surrounded by flattened cells of the outer bulb. Distal phalanx, middle finger. 81 mm. c.r. length. H. & E., 10μ . $\times 1200$.
- Fig. 9. Longitudinal section through the base (B) and the distal end (D) of the distal phalanx showing two branches of the collateral digital nerve with developing Pacinian corpuscles (arrows). 81 mm. c.r. length. H. & E., 10μ . $\times 140$.

PLATE 2

- Figs. 10-13. Four consecutive serial transverse sections of a developing Pacinian corpuscle. Fig. 10, distal end, figs. 11 and 12, middle part, and fig. 13, proximal end, of the corpuscle. Arrows indicate the inner bulb or central core. Distal pad, index finger. 88.8 mm. c.r. length. H. & E., 10μ . $\times 600$.
- Fig. 14. Oblique section of a developing Pacinian corpuscle showing its cellular structure and ramification of the nerve fibre (arrow). N, fascicle of nerve fibres; V, blood vessel adjacent to the corpuscle probably to be later incorporated into its structure. Distal pad, index finger. 95 mm. c.r. length. Simplified Bielschowsky-Gross silver impregnation method. $\times 600$.

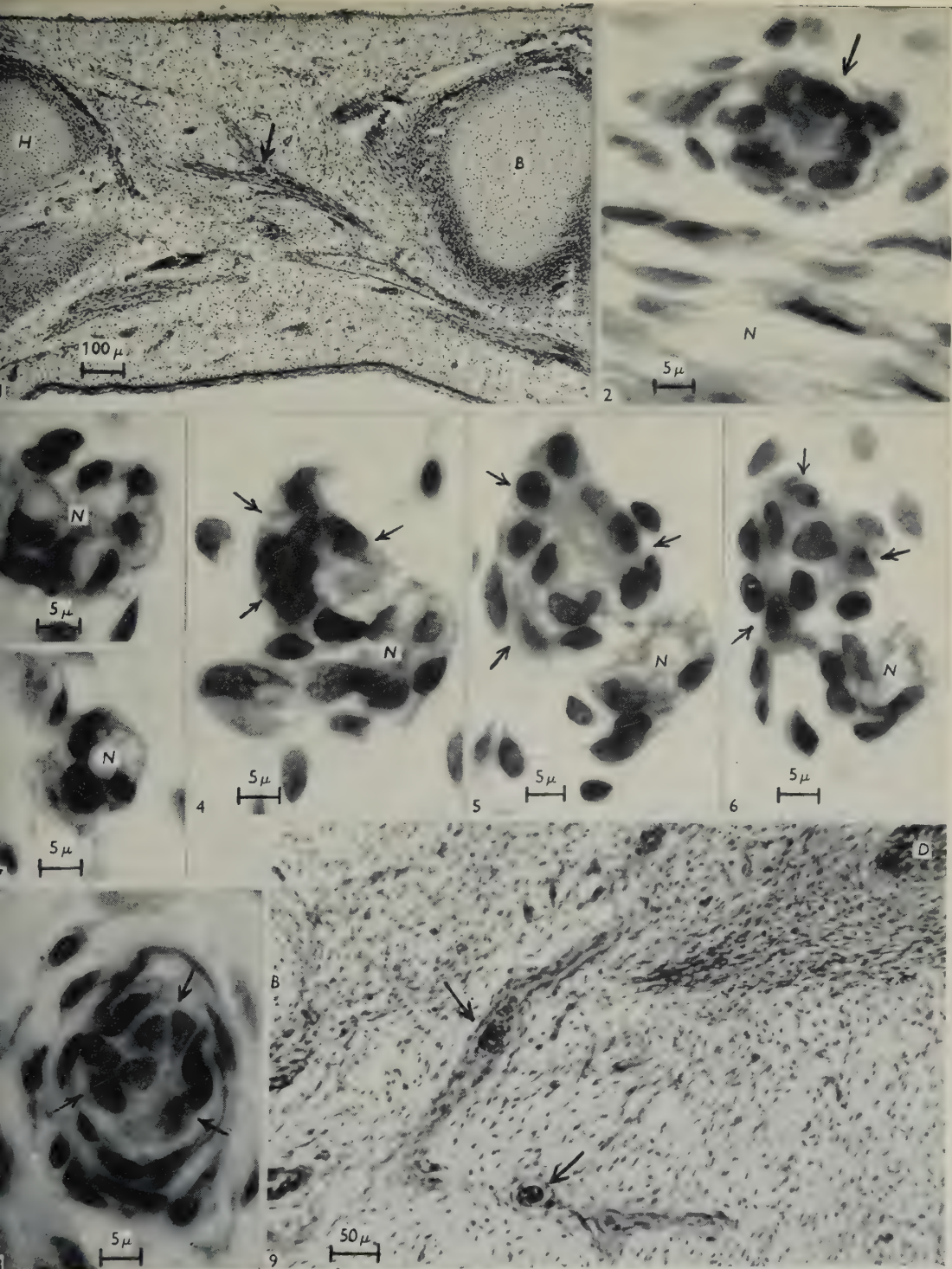
- Fig. 15. Longitudinal section of a developing subcutaneous Pacinian corpuscle (arrows) with the nerve fibre derived directly from a bundle of axons. Distal pad, index finger. 125 mm. c.r. length. Simplified Bielschowsky-Gross silver impregnation method. $\times 600$.
- Fig. 16. Transverse section of a developing Pacinian corpuscle showing its cellular structure. Middle phalanx, middle finger. 95 mm. c.r. length. H. & E., 10μ . $\times 1200$.
- Fig. 17. Oblique section of a developing Pacinian corpuscle showing the inner bulb (arrows) and the early formation of the cellular outer bulb from the surrounding adventitial tissue. Proximal phalanx, index finger. 110 mm. c.r. length. H. & E., 10μ . $\times 600$.

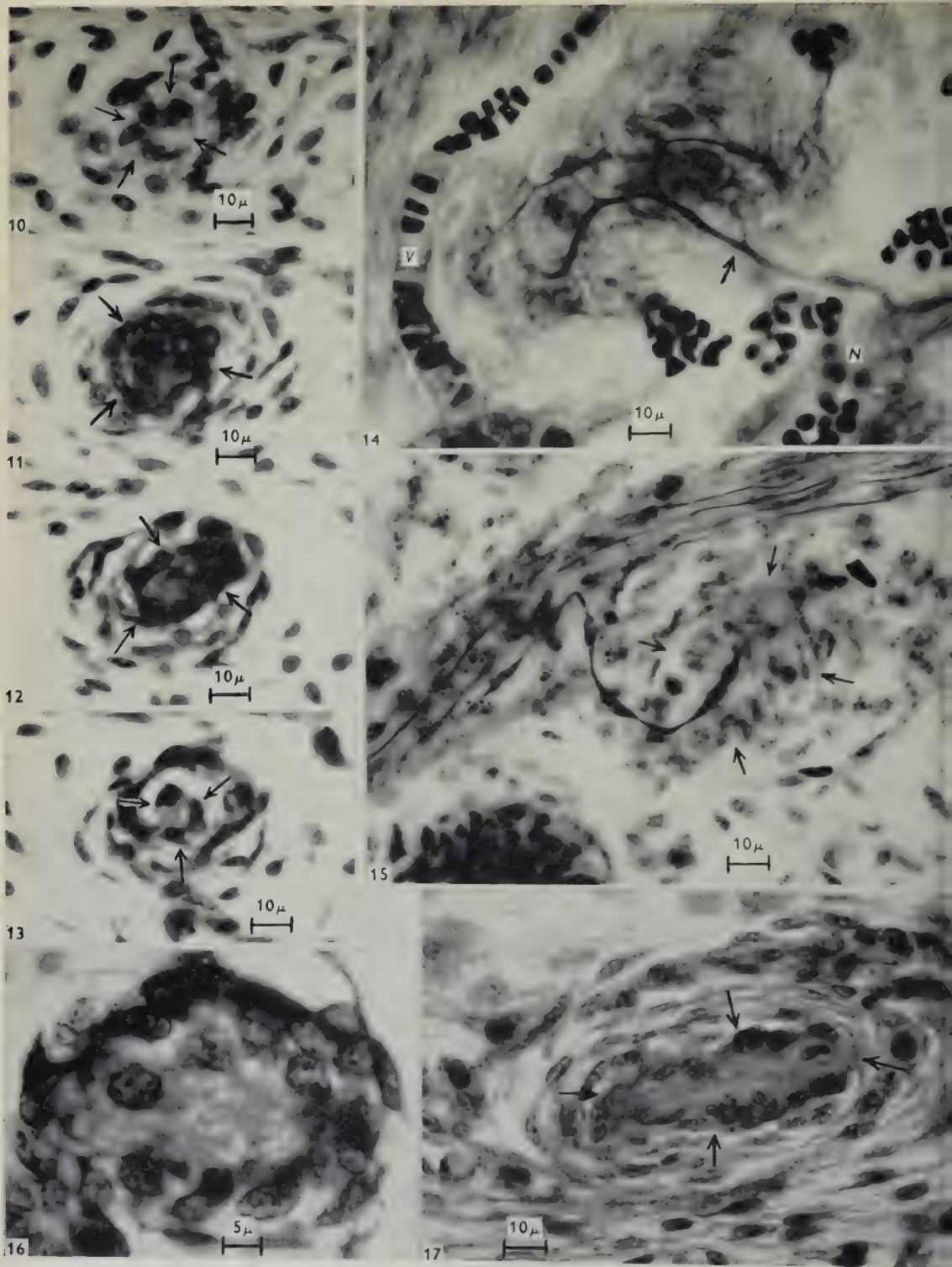
PLATE 3

- Fig. 18. Transverse section of a developing Pacinian corpuscle. Distal pad, ring finger. 145 mm. c.r. length. H. & E., 12μ . $\times 600$.
- Fig. 19. Field from a longitudinal section of the thumb (palmar aspect) showing that the Pacinian corpuscle (arrows) stains yellow with Van Gieson's stain (grey in the black and white photograph) and consists of cells only, like the epidermis (*E*) and sweat glands (*S*). Extracellular fibres staining red (black in the photograph) are not present in the corpuscle at this stage of development. *T*, flexor tendon. 125 mm. c.r. length. 10μ . $\times 140$.
- Fig. 20. Longitudinal section of a branch of the collateral digital nerve and a blood vessel (*V*). An axon of the nerve (arrows) and a branch of the blood vessel are linked together within the developing Pacinian corpuscle (*P*). Middle phalanx, little finger. 144 mm. c.r. length. Simplified Bielschowsky-Gross silver impregnation. Hyalase method. Frozen section, 20μ . $\times 250$.
- Fig. 21. Longitudinal section of a subcutaneous Pacinian corpuscle with the central nerve fibre ending in an enlargement; a bud (arrow) can be seen arising from the side of the stem fibre. Middle phalanx, index finger. Premature foetus, weight 1 kg. Simplified Bielschowsky-Gross silver impregnation method. Frozen section, 40μ . $\times 1200$.
- Fig. 22. Two subcutaneous Pacinian corpuscles cut transversely; a vein (arrows) draining one of them joins the venous end of a developing arteriovenous anastomosis (*AV*). The plane of the section corresponds to the plane indicated in fig. 23. Distal pad, index finger. 204 mm. c.r. length. H. & E., 10μ . $\times 425$.
- Fig. 23. Drawing from a wax-plate reconstruction showing two Pacinian corpuscles and a developing arteriovenous anastomosis (*AV*). Veins draining the corpuscles join the venous end of the anastomosis. The line indicates the plane of the section shown in fig. 22. Distal pad, index finger. 204 mm. c.r. length. Reconstruction represents 42 sections, 10μ each. $\times 170$.

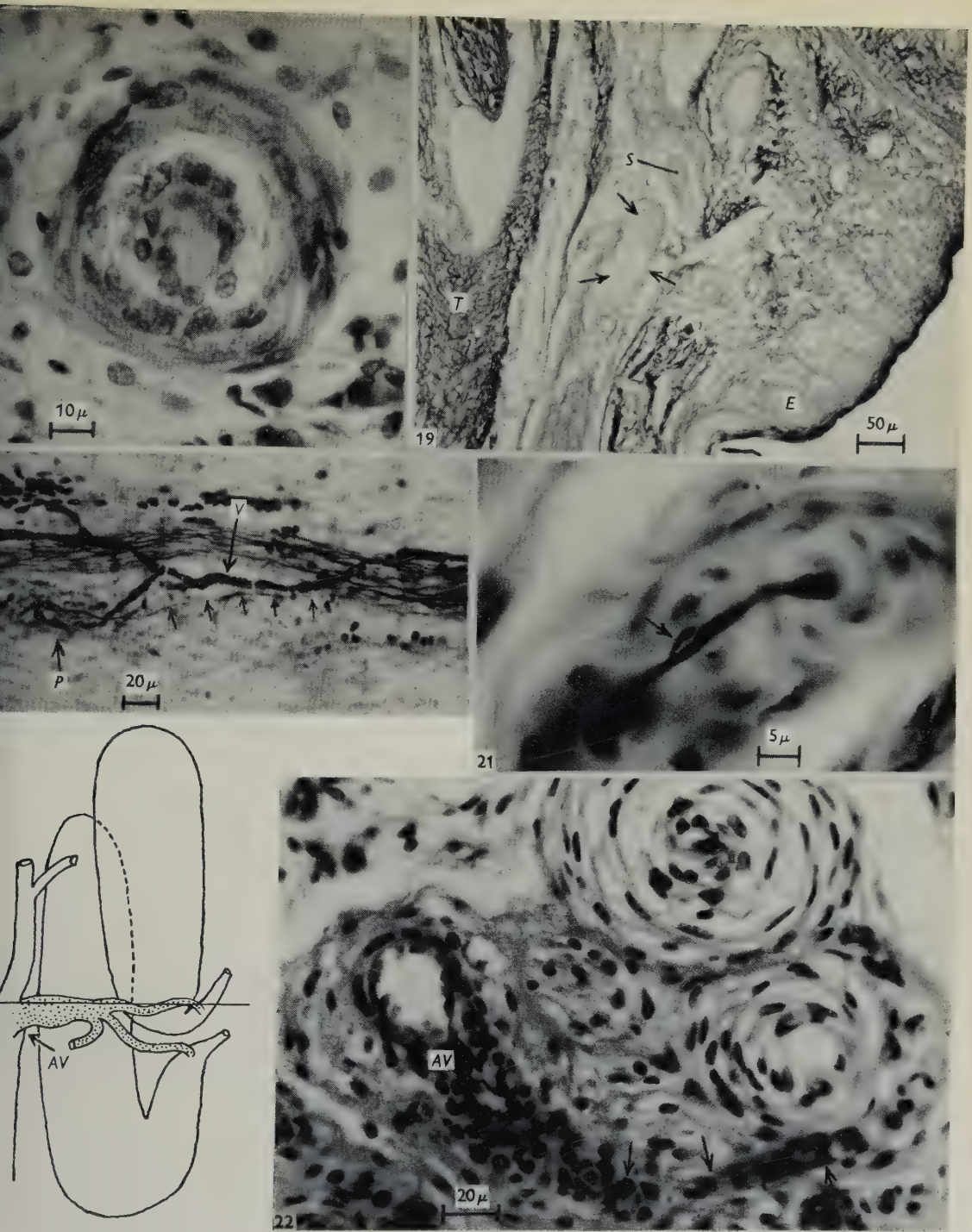
PLATE 4

- Fig. 24. Longitudinal section of the collateral digital nerve and two Pacinian corpuscles. An axon (arrows) supplying one of the corpuscles arises directly from the nerve fascicle. Proximal phalanx, index finger. 125 mm. c.r. length. Simplified Bielschowsky-Gross silver impregnation method. Frozen section, 20μ . $\times 280$.
- Fig. 25. Longitudinal section of two developing periosteal Pacinian corpuscles (arrows) supplied by branches of one axon. Side of the proximal phalanx, index finger. 144 mm. c.r. length. Simplified Bielschowsky-Gross silver impregnation method. Frozen section, 40μ . $\times 250$.
- Fig. 26. Parts of a single coiled Pacinian corpuscle (see Text-fig. 3 for orientation). Distal phalanx, index finger. Male, 22 years. H. & E., 12μ . $\times 140$.
- Fig. 27. Coiled Pacinian corpuscle surrounded by a common lamellar system. Distal phalanx, index finger. Male, 2 years. Simplified Bielschowsky-Gross silver impregnation method. Frozen section, 40μ . $\times 100$.
- Fig. 28. Mesenteric Pacinian corpuscles. The corpuscle on the left is developing a proximal segment. Cat, aged 1 week. Simplified Bielschowsky-Gross silver impregnation method. Whole mount. $\times 120$.
- Fig. 29. Proximal part of a Pacinian corpuscle (*P, P*) and its stalk (*S*) of an infant. The nerve fibre supplying the corpuscle has a myelin sheath (*M*) while traversing the stalk but loses it on entering the corpuscle proper. Distal phalanx, index finger. Male, 2 years. Simplified Bielschowsky-Gross silver impregnation method, 40μ . $\times 600$.
- Fig. 30. Pacinian corpuscle from the mesentery of the cat showing blood vessels surrounding the corpuscle in the form of a basket. Adult cat. Whole mount, light haematoxylin stain. $\times 30$.

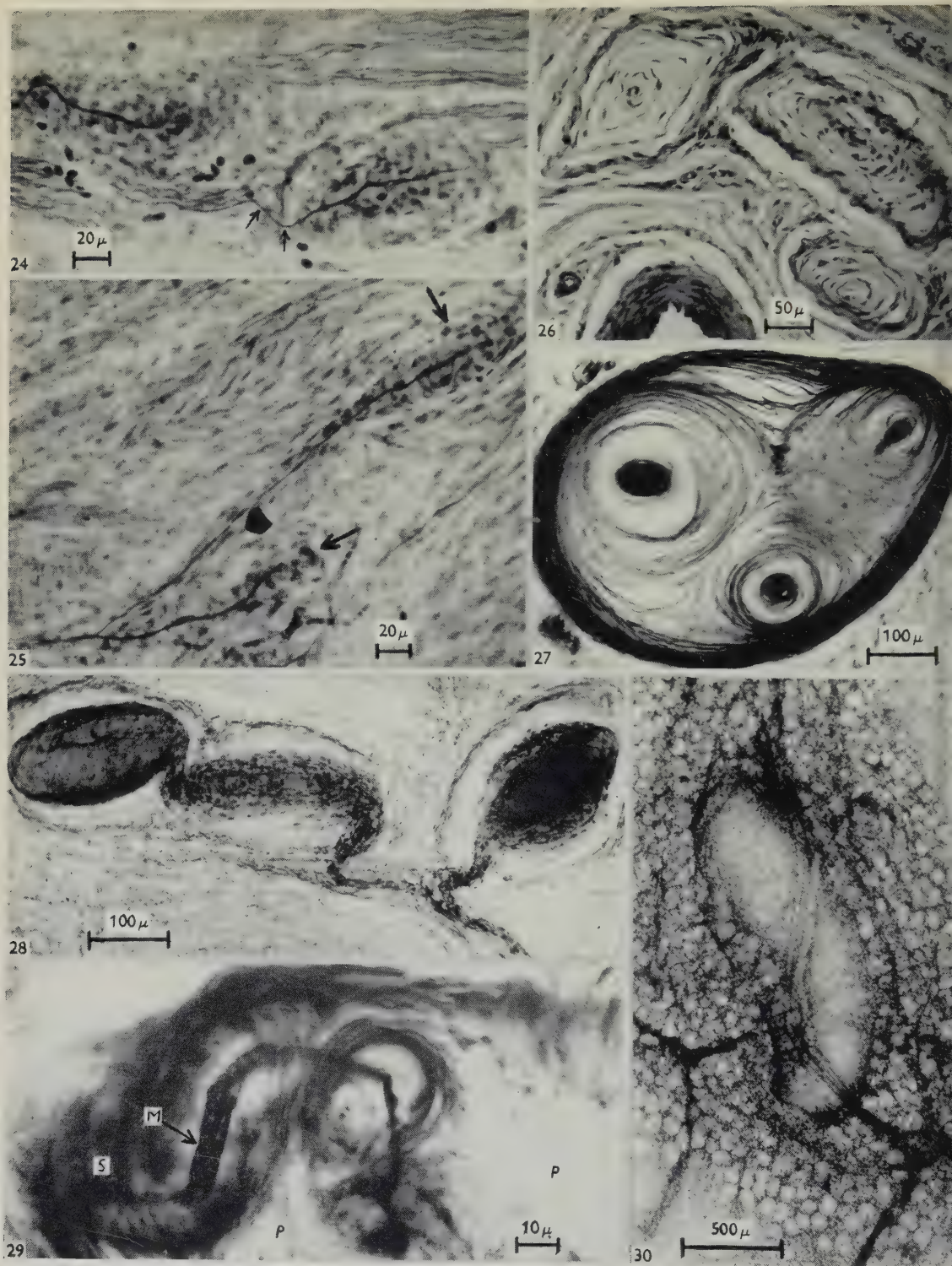




CAUNA AND MANNAN—DIGITAL PACINIAN CORPUSCLES IN THE HUMAN HAND



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CAUNA AND MANNAN—DIGITAL PACINIAN CORPUSCLES IN THE HUMAN HAND

AN ELECTRON MICROSCOPIC STUDY OF THE RAT SUBMAXILLARY GLAND DURING ITS POST-NATAL DEVELOPMENT AND IN THE ADULT

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In a previous paper (Jacoby & Leeson, 1959) a histological account has been given of the post-natal development of the rat submaxillary gland from birth to 6 months. The precise architecture of the adult gland was detailed also, and an attempt made to clarify the confusing terminology existing in the literature concerning the various epithelial components of this gland.

It was found that acini are not present at birth. The most peripheral elements of a glandular unit are branching terminal tubules which are linked to an intercalated duct; and this, in turn, is continuous with an intralobular striated duct. The cells of the terminal tubules contain fine acidophil granules. At the circumference of the terminal tubules, from the first week on, cell 'buds' and crescents appear, which, by proliferation and differentiation, become the acini. The terminal tubules are gradually reduced in size, lose the granules and become transformed into intercalated ducts, second order. This process is completed by approximately the sixth week, by which time the intralobular striated ducts have, as the result of mitotic activity, increased considerably in extent and have become convoluted. The next phase is characterized by further growth of these convoluted ducts and by an elaboration, within their cells, of circumscribed secretion granules. This granulation reaches massive proportions, and in this way the convoluted striated intralobular ducts become, with the exception of their most distal segments, transformed into 'granular' tubules.

The present study deals with the electron microscopic (E.M.) appearance of the main epithelial elements of the rat submaxillary gland as seen in some selected stages during its post-natal development and in the adult. Such a study not only reveals the fine structure of the various epithelial cells, but also helps in clarifying controversial issues which have arisen in the past and which could not be settled by light microscopy (L.M.).

MATERIAL AND METHODS

Rats aged 1 day, 2, 4 and 6 weeks and 6 months were used. They were killed by a blow on the head, and the submaxillary glands dissected out. These were cut into small cubes, approximately 1 mm.³ in size, and placed in fixative within 2 min. of death.

The fixative used was 1 % osmium tetroxide buffered to pH 7.2 either with acetate veronal (Palade, 1952) or bichromate (Dalton, 1955). Fixation times varied from 30 to 60 min. The specimens were then washed in distilled water and dehydrated through

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graded alcohols. Following infiltration with several changes of 5% methyl in *n*-butyl methacrylate, they were embedded in this medium in gelatine capsules, and polymerization was effected at 60° C. for 18 hr. At this temperature polymerization damage is minimal (Borysko, 1956). 1% benzoyl peroxide was used as catalyst.

Blocks were trimmed to give an initial block face of 0.2 mm.² Sections were cut with glass knives on a heat advance ultra-microtome (Sims & Leeson, 1957), collected on carbon-coated grids and examined in a Metropolitan-Vickers EM3 at 75 kV.

RESULTS

Day 1. Terminal tubules (Pl. 1, fig. 1): the epithelial cells are arranged around an irregular relatively wide (4–6 μ diameter) lumen. They are bounded by a distinct cell membrane. From the apices scattered microvilli project which vary in number and size from tubule to tubule. They may be up to 0.8 μ in height and 0.1 μ in width. They are more frequently seen on cells which contain only a few apically situated secretion granules. The cell nucleus is basally situated, pale and contains several nucleoli of great electron density. Aggregations of paired membranes (endoplasmic reticulum (E.R.)* of Palade & Porter, 1952, or α -cytomembranes of Sjöstrand, 1956) are present mainly in the basal half of the cell and show a strikingly parallel arrangement. There are a few mitochondria, mostly near the nucleus; they are elongated, sometimes branched, with an internal pattern of membranes or cristae. The apical halves of the cells contain a varying number of dense spherical granules, differing in size from 0.5 to 1.5 μ . Some cells have only very few such granules, others are almost filled with them. The rest of the cytoplasm has a moderate electron density and appears finely particulate.

Some terminal tubules have associated with them one or two cells which do not quite conform to this description; they tend to have a darker nucleus and 'secretory' inclusions which are larger, paler and less well circumscribed than the granules mentioned above (Pl. 1, fig. 1). These cells are thought to be identical with the 'budded' cells observed with the L.M. (Jacoby & Leeson, 1959) and interpreted as being immediate precursors of acinar cells.

Intercalated ducts, i.e. the smallest intralobular ducts observable, have flat cells, largely occupied by nuclei with marked indentations (Pl. 1, fig. 2). The dense nucleoli are irregular in outline. Oval mitochondria are scattered throughout the dense and finely particulate cytoplasm. The cell membrane covers minute microvilli here and there on the luminal surface and shows interlocking between adjacent cells, forming meandering outlines (Pl. 1, fig. 3), but no basal infoldings. There are no secretory granules nor an E.R.

Larger intralobular ducts: these are lined by columnar cells with pale, round or oval nuclei containing one or two dense nucleoli, which are less irregularly shaped than those seen in the nuclei mentioned above. The general cytoplasm is paler and has more scattered particles than that of the intercalated ducts. Mitochondria, ovoid in shape, are more numerous than in terminal tubules and intercalated ducts

* In this paper the term E.R. is used only with reference to intracellular granular (or rough) paired membranes.

and are aggregated below and just above the nucleus. The basal cell membrane shows short but definite infoldings. There is no E.R.

In both the intercalated and the larger intralobular ducts an occasional extra basal cell is seen, lying inside the basement membrane, with its nucleus elongated parallel to the circumference of the duct (Pl. 1, fig. 2). Such a nucleus may well be that of a myo-epithelial cell.

2 weeks. *Acinar cells*, occasionally seen isolated, but more frequently as 'buds' or crescents of terminal tubules, are now clearly differentiated (Pl. 1, fig. 4). Their two most striking features are: (1) great electron density of the basal nucleus which often makes it difficult to distinguish a nucleolus within it, and (2) large pale rounded 'secretory' material, which is incompletely delineated by a membrane and thus coalesces into formations reminiscent of 'cumulus clouds' (Pl. 2, fig. 5). There is an aggregation of double membranes (E.R.) running parallel, curved or straight, below and around the nucleus. Mitochondria are few and far between and mainly arranged peripherally alongside the cell membrane. This membrane shows some meandering at the interfaces of neighbouring cells. Here, occasionally, the two cell membranes are separated over a short distance, enclosing a definite space, i.e. an intercellular 'secretory' capillary (Pl. 2, fig. 5).

The cells of terminal tubules, centrally placed in relation to the acinar cells or crescents (Pl. 1, fig. 4), show only minor changes in appearance compared with those of the earlier stage: microvilli are reduced in number and size, and 'secretory' granules are less numerous, smaller and often concentrated apically.

The fine structure of the intercalated ducts is unaltered.

The intralobular 'striated' ducts now show deep infoldings of the basal cell membrane. Many of these folds extend up to the nucleus which is situated centrally in the cell. They are rarely straight, but often turn back on themselves producing undulating loops (Pl. 2, fig. 6). Between and alongside these folds are numerous elongated mitochondria often with their long axes arranged parallel to the long axis of the cell. Mitochondria are also found around and above the nucleus, but are less frequent in cell apices.

4 weeks (Pl. 2, figs. 7, 8). The acinar cells are filled with a large amount of secretory material, which is even more cloud-like and ill circumscribed. The narrow strip of basal cytoplasm contains the dense nucleus and rows of double membranes (E.R.) either lying parallel to the basal cell membrane or curving away from it. Intercellular secretory capillary spaces are seen which often show invaginations into the side of the cells.

Terminal tubules are much reduced in extent and appear as 'centres' which on account of their great electron density stand out strikingly against surrounding crescents of pale acinar cells (Pl. 2, fig. 7). This contrast is enhanced by the presence, in the cells of the terminal tubules, of numerous and often large secretion granules, most of which are very dense and all of which are sharply outlined. A basal E.R. is present. Microvilli are no longer present either in terminal tubules or in intercalated ducts; only short stumps are occasionally seen.

Intralobular striated ducts. Mitochondria with their characteristic internal pattern are more numerous, and many of them are elongated. Their distribution and arrangement is as before. The same can be said of the infoldings of the basal cell membrane.

The cell apices sometimes bulge slightly into the lumen and are covered by a crenated cell membrane. The apical regions are pale and contain, besides a few mitochondria and scattered particles, some minute vesicles (up to 1μ).

6 weeks. Only remnants of terminal tubules or 'centres', sometimes in the form of a single cell, are present. Some of these 'shrunk' cells still contain a few dense granules, others are quite devoid of granules, showing instead a few small vacuoles (Pl. 3, fig. 9); they are also devoid of an E.R., having become transformed into cells of an intercalated duct, second order (Jacoby & Leeson, 1959). The acinar cells are more or less fully developed. The cloud-like ill-defined secretion material practically fills the cell, but in a tangential section through the basal region the E.R. is well displayed (Pl. 3, fig. 10). Intercellular spaces (secretory capillaries) are evident. Intralobular 'striated' ducts show a still greater number of mitochondria, rather closely packed in the basal half of the cell in between complex infoldings of the basal cell membrane (Pl. 3, fig. 11). In some cell apices there are now found definite small, well circumscribed granules of great electron density (Pl. 3, fig. 12). Very short stumpy microvilli were seen on the luminal surface of cells of two such ducts.

6 months. The appearance is now that of a fully developed adult gland. In order to avoid repetition, only salient features will be considered. The cell membranes of acinar cells, though smooth at the base, show undulating folds at interfaces with neighbouring cells (Pl. 4, fig. 14) and commonly enclose intercellular secretory spaces. The pale cloud-like secretion material shows a stippled network inside (Pl. 4, fig. 15). It is hardly ever bordered by a distinct membrane, but merely surrounded by so-called 'Palade granules' or, if seen in the basal part of the cell, it is found to lie in closest proximity to the E.R. The great electron density of the nucleus is striking.

The cells of *granular tubules* (Pl. 4, fig. 16)—in accordance with their L.M. appearance—are more or less filled, except for the basal region, with sharply circumscribed granules varying in size from 0.5 to 2μ or more, the larger granules predominating. Most of them are dense and dark, surrounded by an even denser fine line. Some are pale, and then the surrounding line stands out more clearly. The mitochondria are mainly concentrated near the base where also the nucleus is located. The basal cell membrane shows definite infoldings, which, however, do not extend very high up and which are not very complex. Elongated mitochondria often lie parallel to these folds.

Striated ducts. In contrast to the rudimentary infoldings of granular tubules, the infoldings of the basal cell membrane of striated ducts are frequent, high and complex (Pl. 3, fig. 13; Pl. 4, fig. 17). Similar meandering folds are also a feature of intercellular cell membranes, but only along the lower third of the cell (Pl. 4, fig. 17). Very short microvilli are sometimes present on the luminal cell surfaces. Numerous mitochondria are aggregated between the basal infoldings. The apical region is pale and devoid of secretion granules (Pl. 4, fig. 18). Fine dense particles and very small vesicles form here a background to scattered mitochondria. Short double membranes with attached granules (E.R.) are present in the basal region of the cells, but do not form an extensive system (Pl. 4, fig. 17).

DISCUSSION

The observations made with the E.M. on these selected post-natal stages of developing rat submaxillary glands are both confirmatory and supplementary to our previous findings with the L.M. The presence of secretory granules in the cells of terminal tubules and, later, in those of convoluted granular tubules belongs to the first category. The differences in electron density of these granules, which parallel differences in staining intensities, could indicate differences in degree of granule maturation. At the 2 weeks' stage we have, under L.M., recorded a paler staining of the cells of terminal tubules; now we find less striking granulation with the E.M. Also, the massive granulation seen with the E.M. at 4 weeks in the cells of terminal tubules has its counterpart in the most intense staining observed at this same stage with the L.M. Again, the first signs of elaboration of definite secretion granules in the apices of cells of intralobular striated ducts were seen, with the E.M., at the 6 weeks' stage which also agrees well with the L.M. findings.

The points brought out by the E.M. which are supplementary to the L.M. observations are of special interest. There is a definite E.R. in the basal region of the cells of terminal tubules, which is only present when they elaborate granules. When the 'reduced' cells of terminal tubules or 'centres' have ceased to do so and have become transformed into intercalated duct cells, they no longer show an E.R. On the other hand, terminal tubular cells are the precursors of acinar cells which, in turn, show a very rich E.R. Since the work of Dalton, Kahler, Striebich & Lloyd (1950) and Dalton (1951) on liver, pancreas and stomach, and of Bernhard, Hagenau, Gautier & Oberling (1952) on liver, pancreas and salivary glands, the intracellular membranes or lamellae, now widely called E.R., have been equated with the basophilic 'Basallamellen' of Heidenhain or Garnier's ergastoplasm. Gautier and Diomedes-Fresa (1953), who studied rat submaxillary glands, observed in acinar cells an ebb and flow of E.R. in relation to secretory phases corresponding to changing degrees in basophilia. They aptly compared the pattern of the E.R. in these cells to that of 'finger prints'. We can confirm the correspondence of E.R. and basal chromidial substance as far as acinar cells are concerned, but as to terminal-tubular cells such basophilia was less evident, though not entirely lacking.

A functional relation between E.R. and elaboration of secretory material can easily be construed with regard to the acinar cells and the cells of the terminal tubules, but such a hypothesis fails to be applicable to the granular tubules, where in spite of massive elaboration of what are apparently secretory granules there is no E.R.

The pale cloud-like secretion material in acinar cells needs some special comment: this material is never completely bounded by a membrane; in fact, it becomes more and more ill-defined as time goes on, and the individual round masses fuse with one another. There is then no evidence, from these E.M. studies, for the existence of secretion *granules* in these cells, which has been a controversial point of long standing.

The dark (electron dense) nucleus of the acinar cells is surprising in view of its appearance under the L.M. This nuclear density may be related to, and concerned with, the existence and the maintenance of the extensive E.R.

So-called secretory capillaries are clearly present between acinar cells, and only between these. They are always strictly intercellular, and even where they indent

the sides of a cell or appear to penetrate it, they are always delineated by the cell membrane. Thus there is no evidence for the existence of truly intracellular canals such as, prior to the E.M. era, have been repeatedly described (e.g. Krause, 1895; see also Zimmermann, 1927). Secretory capillaries of this kind have been said to be characteristic of serous cells only (see Schaffer, 1927), but in rat submaxillary glands they are present between acinar cells which, on account of their staining reactions (Jacoby & Leeson, 1959), are, if anything, more mucous than serous.

Microvilli were found in terminal tubules, intercalated and striated ducts, at almost all stages. They usually were seen to be very short and stumpy, hardly deserving the name; or else, from 4 weeks on, they were absent altogether. They had a commendable height only in the terminal tubules at day 1. We are unaware of their significance at this stage. Our observations show a considerable decrease with time in height of the microvilli until they are rudimentary stumps. This could indicate that they came originally into being (e.g. in the terminal tubules) when lumen formation took place possibly as a result of vigorous cell movements.

Finally, the specializations of the intercellular and basal cell membranes have to be discussed. Moderate folding and interlocking of *intercellular* cell membranes were noticed amongst acinar cells, more intricate ones in intercalated ducts, and distinctly elaborate ones between cells of the striated ducts, where they were found to be confined to the lower third of the epithelium. This, on the whole, agrees with the findings of Pease (1956). The most consistent, striking and extensive infoldings, however, are those of the *basal* cell membrane of the striated ducts. Such infoldings were first discovered in various epithelia of the kidney (Sjöstrand & Rhodin, 1953; Rhodin, 1954). This specialization results in an enormous increase in area of the basal cell membrane, and this has been linked up with a free and fast transport of water and ions. Pease (1956) studied with the E.M. various epithelia known to transport water rapidly, such as ciliary epithelium, that of the choroid plexus and that of ducts of salivary glands, using rat submaxillary gland. He found that such infoldings, though differing in configuration, were present in all three types. He illustrates particularly well what we have called the meandering course taken by these folds.

Water transport may well be one of the earliest functional activities of salivary glands, and it is noteworthy that basal infoldings, though less complex, are already present at birth, both in the inter- and the larger intralobular ducts, and that with the L.M. striations were seen in these ducts at the same stage. In this connexion it is not only of interest, but most appropriate to recall Pflüger's (1866) description of these structures. He is credited with the discovery of the striations; he wrote: 'Das Wunderbarste an diesem Cylinderepithel ist die dem Lumen abgekehrte Seite. Hier entspringen unendliche Mengen der allerfeinsten, immer varikösen Härchen, so dass die Oberfläche des sich leicht isolierenden Schlauches wie eine dichte Bürste aussieht.' This morphological analysis is certainly remarkable, though the bristles he saw may have been aggregates of infoldings. He considered this 'brush' to be the explanation of the striation. Subsequently, especially after the discovery of mitochondria, Pflüger's contention was lost sight of, and Regaud & Mawas wrote in 1909: 'L'alignement de ces éléments (sc. mitochondria) en séries est la cause de la

striation'. It seems now that both the deep infoldings of the basal cell membrane and the mitochondrial array alongside the folds combine to produce the effect of striation as seen with the L.M.

SUMMARY

1. Submaxillary glands of rats aged 1 day, 2, 4, 6 weeks and 6 months were studied with the electron microscope.

2. The cells of terminal tubules have discrete dense secretion granules apically and basally a system of double membranes (endoplasmic reticulum (E.R.)) and scattered elongated mitochondria within a generally dense and finely particulate cytoplasm. The cell content of secretory granules is most marked at 4 weeks. Thereafter the cells, which eventually become cells of intercalated ducts, 2nd order, lose both granules and E.R.

3. The acinar cells, which originate as 'buds' from terminal tubules, are characterized by nuclei of great electron density, a rich E.R., both these being situated basally; and pale 'cloud-like' secretion material occupying the bulk of the cell. This material is poorly circumscribed and becomes more so as time passes. Strictly intercellular secretory capillaries are present between the acinar cells.

4. The intercellular cell membranes show meandering folds and interlocking between acinar cells, between intercalated duct cells and, in the basal third only, between cells of striated ducts.

5. Infoldings of the basal cell membrane are present in intralobular ducts (intercalated ducts excepted) from the earliest stage on, differing in degree according to duct segment and age. Numerous elongated mitochondria lie between these infoldings.

6. Microvilli, of any size, were seen only in terminal tubules at day 1.

7. The E.M. findings are discussed as such and in relation to observations by light microscopy.

This work was carried out during the tenure by one of us (C.R.L.) of a British Medical Association Ordinary Research Scholarship. We wish to thank Prof. J. S. Baxter for his criticisms of the manuscript; Mr J. Taylor for the supply of carbon films; and Mr W. Henderson for his valuable technical assistance with the electron microscope.

Addendum. In our previous paper (p. 201 of this volume) a printing error occurred in Table 1. Instead of + + + + + read + - - + + +.

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EXPLANATION OF PLATES

All figures are electron micrographs of rat submaxillary glands.

Abbreviations

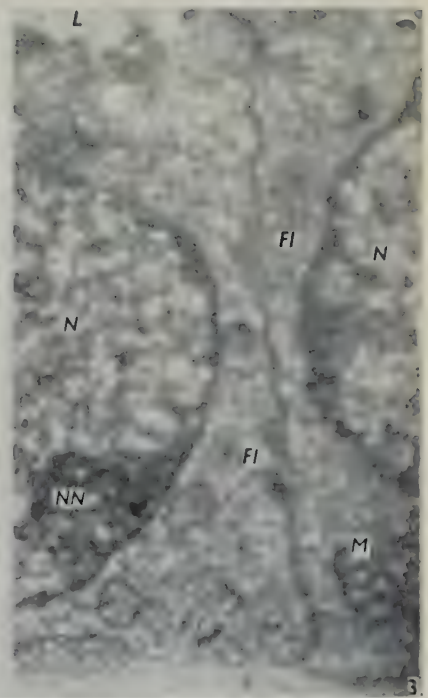
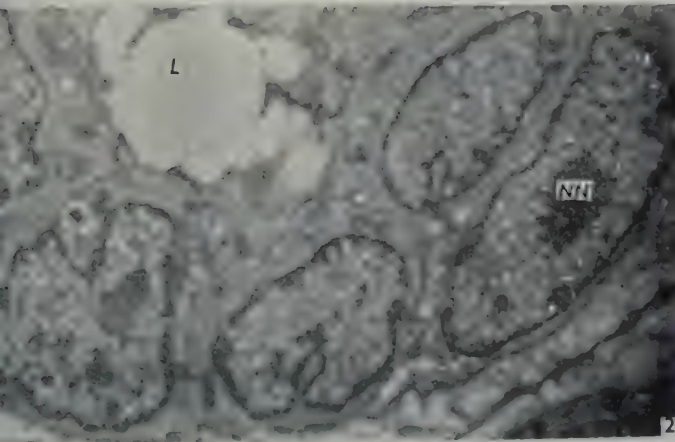
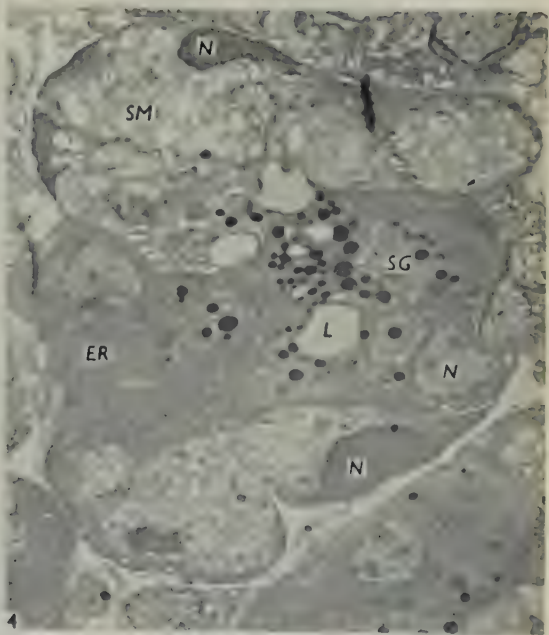
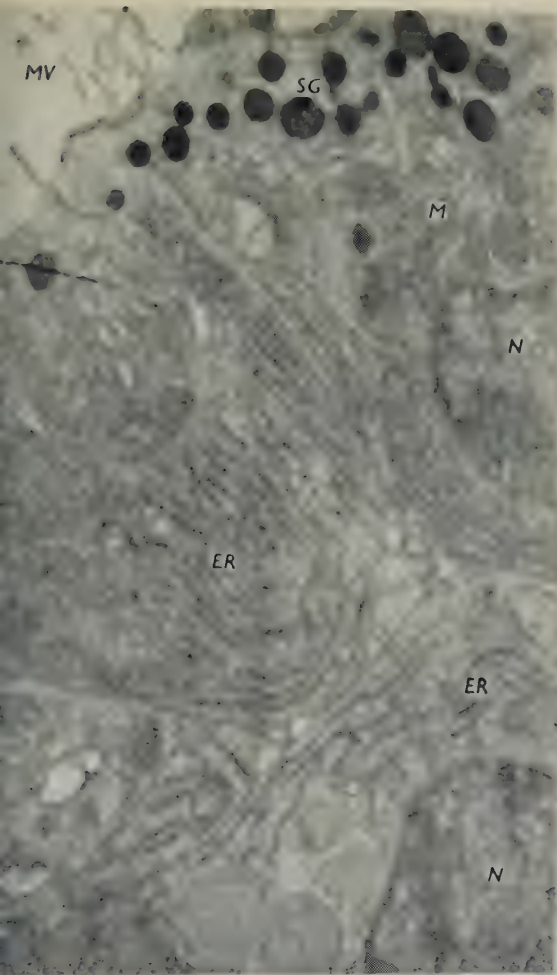
<i>ER</i> = endoplasmic reticulum	<i>N</i> = nucleus
<i>FB</i> = infoldings of basal cell membrane	<i>NN</i> = nucleolus
<i>FI</i> = foldings of intercellular cell membrane	<i>SC</i> = secretory capillary
<i>L</i> = lumen	<i>SG</i> = secretion granules
<i>M</i> = mitochondrion	<i>SM</i> = secretion material
<i>MV</i> = microvilli	<i>TT</i> = terminal tubule

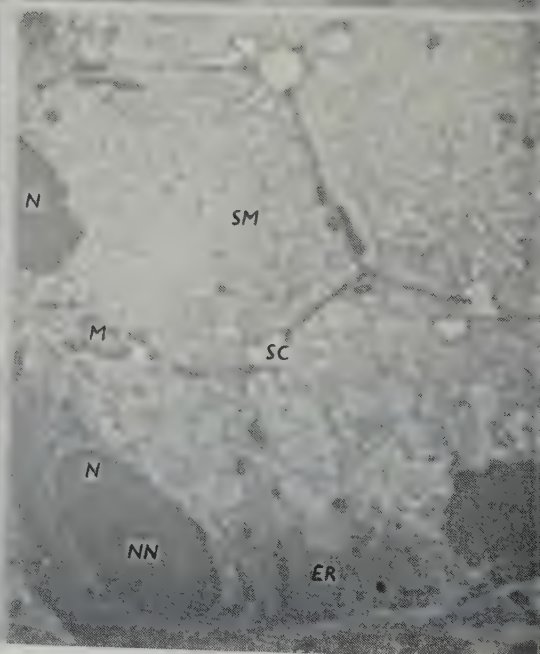
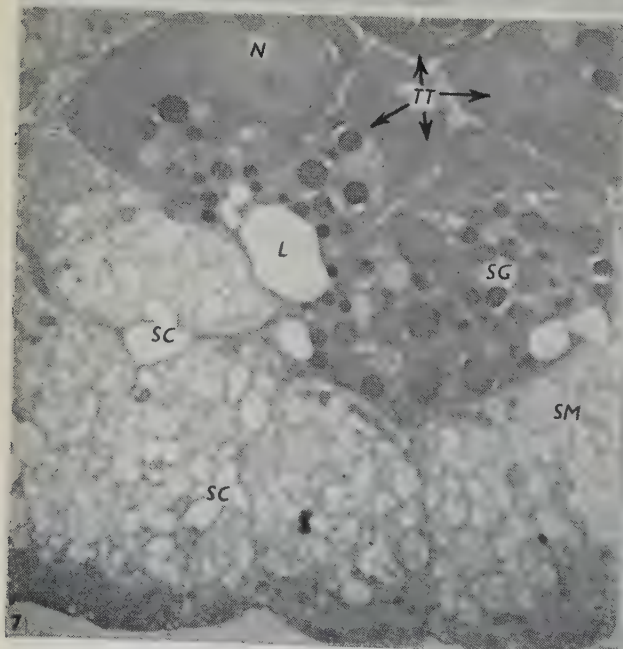
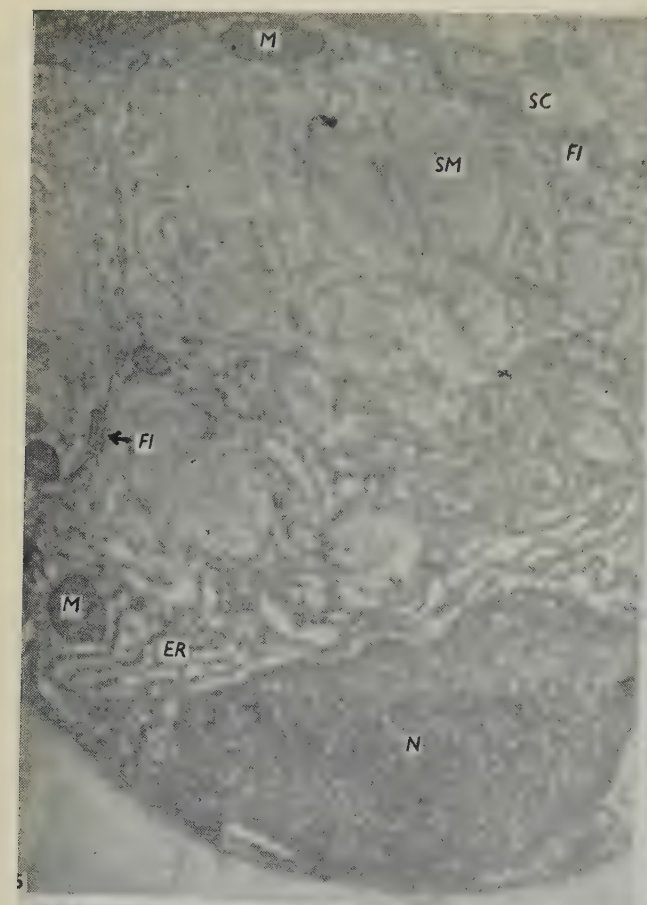
PLATE 1

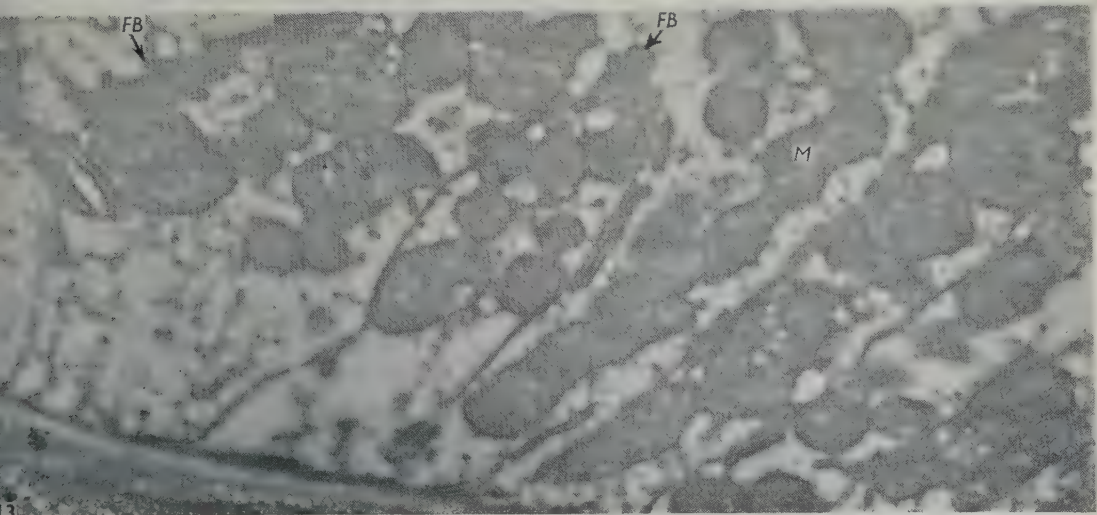
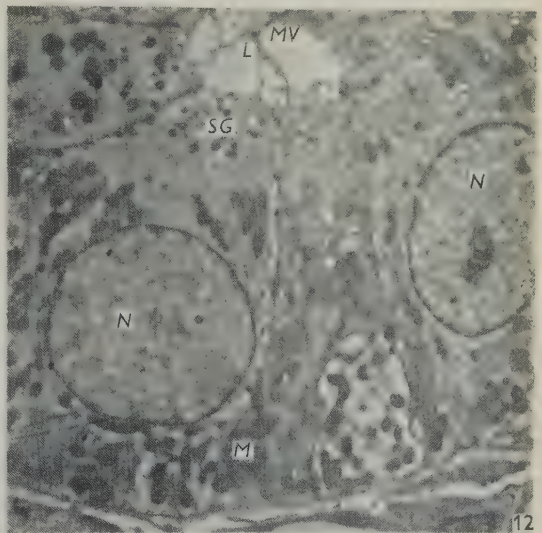
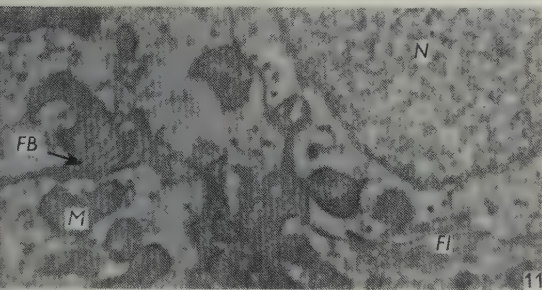
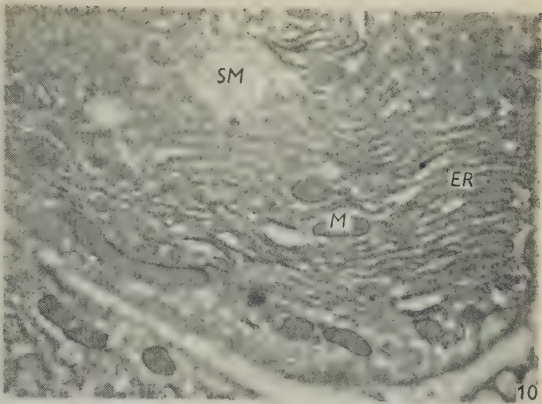
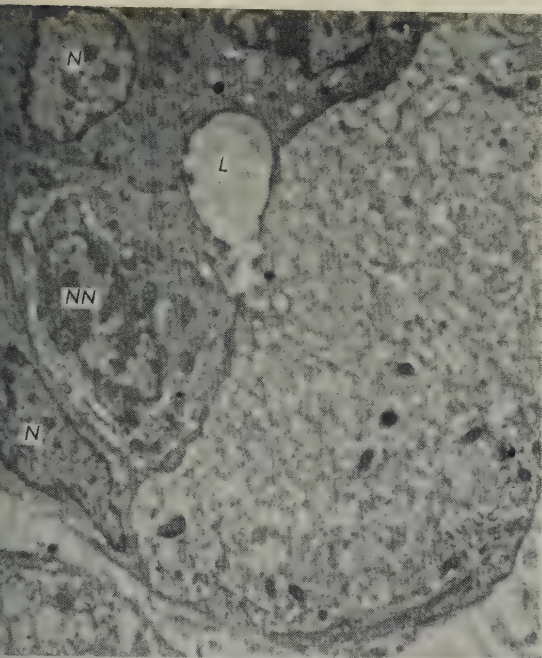
- Fig. 1. 1 day after birth. Cells of a terminal tubule showing microvilli, secretion granules, mitochondria, part of a nucleus and endoplasmic reticulum. The cell in the lower right corner contains larger and paler secretion material. For interpretation see text. 60 min. Palade, $\times 12,000$.
- Fig. 2. 1 day. T.S. of portion of intercalated duct. Note marked indentations of nuclei. To the right, extra basal cell nucleus (? myo-epithelial cell). 45 min. Palade, $\times 7000$.
- Fig. 3. 1 day. Portions of two intercalated duct cells to show 'meandering' and interlocking of the intercellular cell membranes. 60 min. Palade, $\times 15,000$.
- Fig. 4. 2 weeks. Portion of terminal tubule or 'centre' (dense cytoplasm; distinct secretion granules, not very numerous; relatively pale nuclei; *E.R.* and lumen) and acinar crescents. Note in these, dense basally situated nuclei and pale ill-circumscribed secretion material. 60 min. Palade, $\times 2000$.

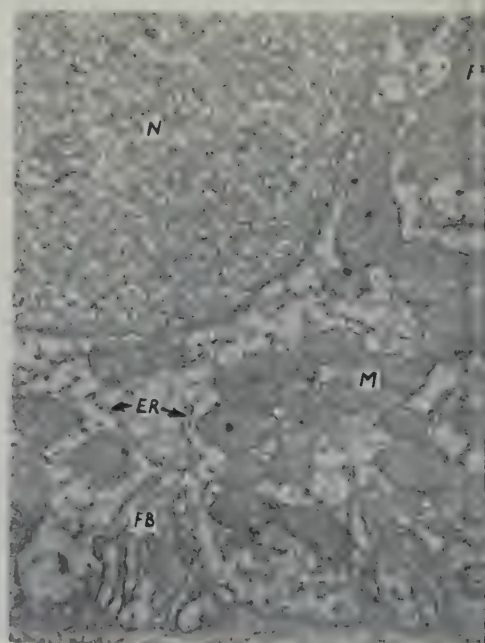
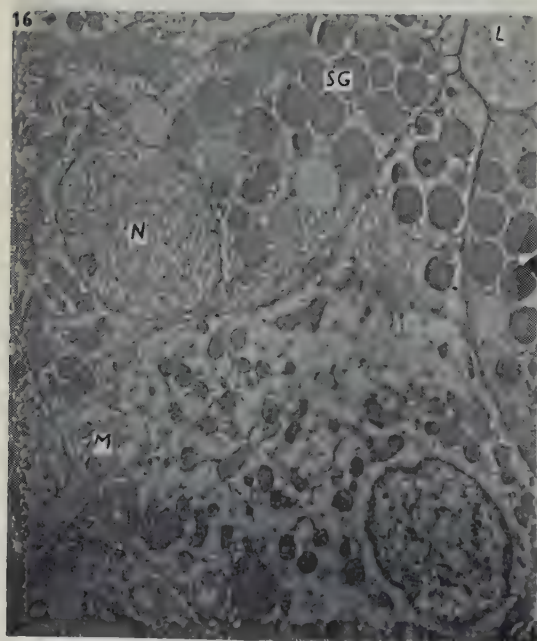
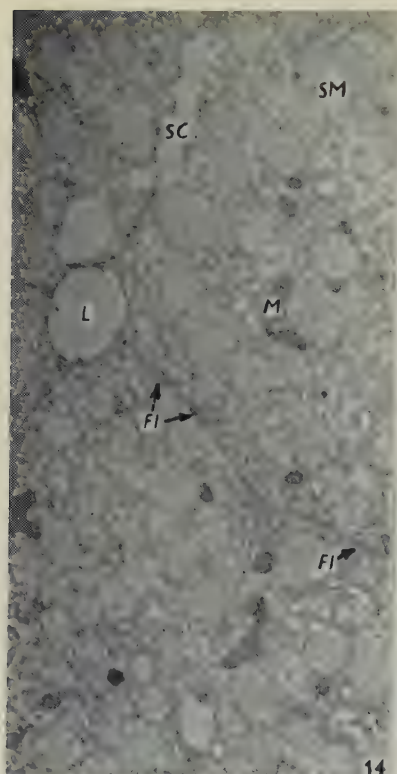
PLATE 2

- Fig. 5. 2 weeks. Portions of three acinar cells. Note cloud-like, partly outlined secretion material, basally situated dark nucleus, *E.R.*, peripherally situated mitochondria, and folding of the intercellular cell membranes which, at one point, enclose an intercellular space (secretory capillary). 45 min. Palade, $\times 13,000$.









- Fig. 6. 2 weeks. Basal region of intralobular striated duct, basement membrane to the right, to show high infolding and meandering of the basal cell membrane and mitochondria in between the invaginations. 60 min. Dalton, $\times 22,500$.
- Fig. 7. 4 weeks. Part of a 'centre' and of an acinar crescent. Note number and size of secretion granules in the cells of the centre (compare with Fig. 4) and several secretory capillaries between the acinar cells. 60 min. Dalton, $\times 3000$.
- Fig. 8. 4 weeks. Acinar cells. Note dark nuclei, basal E.R., peripherally situated mitochondria, intercellular spaces and ill-defined secretion material. 45 min. Dalton, $\times 4000$.

PLATE 3

- Fig. 9. 6 weeks. Junction of an acinar cell with cells of an intercalated duct, 2nd order (a former 'centre'). Note absence of granules and of E.R. in the latter. A few stumpy microvilli project into the lumen. The nucleus to the left may be that of a myo-epithelial cell. 60 min. Palade, $\times 4000$.
- Fig. 10. 6 weeks. Tangential section through the base of an acinar cell to show the parallel arrangement of the rough double membranes (E.R.) and their close spatial relationship to the secretion material. 30 min. Palade, $\times 12,000$.
- Fig. 11. 6 weeks. Portion of a cell of an intralobular striated duct, basement membrane to the left. Note complex infoldings of the basal cell membrane and similar meandering of the intercellular cell membranes near the right bottom corner. 60 min. Palade, $\times 16,000$.
- Fig. 12. 6 weeks. T.S. of portion of a striated duct showing the earliest signs of transformation into a granular tubule, viz. accumulation of small, dense, discrete secretion granules in the cell apices. Note also the stumpy microvilli, infoldings of the basal cell membrane, and mitochondria. 30 min. Palade, $\times 6000$.
- Fig. 13. 6 months. Vertical section through the basal region of a cell of an intralobular striated duct to show, at several places, the remarkably regular undulations of the infolded basal cell membrane and the numerous mitochondria in between. 45 min. Palade, $\times 21,000$.

PLATE 4

- Fig. 14. 6 months. Portions of the apical regions of four acinar cells. Note lumen, intercellular secretory capillary, pale, ill-defined secretion material, scattered mitochondria and especially the meandering shallow folds of the intercellular cell membranes. 60 min. Palade, $\times 7000$.
- Fig. 15. 6 months. Portions of two acinar cells to show the stippled interior of the secretion material and the close spatial relationship of the latter to the E.R. (the dark irregular spot almost in the middle of the picture is an artifact). 30 min. Dalton, $\times 15,000$.
- Fig. 16. 6 months. T.S. of sector of fully differentiated granular tubule showing numerous large secretion granules above the nucleus. Compare with Fig. 12. 45 min. Palade, $\times 4000$.
- Fig. 17. 6 months. Portions of two adjacent cells of an intralobular striated duct, basement membrane at the bottom. Note particularly the complex foldings of the intercellular cell membranes; also the scattered 'rudimentary' E.R. 60 min. Dalton, $\times 18,000$.
- Fig. 18. 6 months. Apical and middle portion of a cell of an intralobular striated duct. Note absence of secretion granules. Supra- and circumnuclear aggregation of mitochondria. 30 min. Dalton, $\times 6000$.

THE CORACO-CLAVICULAR JOINT

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INTRODUCTION

English anatomical text-books, describing the coraco-clavicular ligament, usually mention that a bursa occupies the interval between its conoid and trapezoid parts (e.g. Johnston & Whillis, 1954; Wood Jones, 1953). Schafer, Symington & Bryce (1915) add that a complete joint is occasionally found here. Joints in this situation are known to radiologists and orthopaedic surgeons who consider them to be rare anomalies. Thus, Moore & Renner (1957) found 5 joints in 3886 roentgenograms, Nutter (1941) found 12 in 1000 roentgenograms, and Wertheimer (1948) found 2 in 277 roentgenograms. All of these cases were detected radiologically and their recognition depended on the presence of a bony process projecting from the clavicle in the region of the conoid tubercle and articulating with the coracoid process, an appearance similar to that shown in Pl. 1, fig. 9. Other single cases found radiologically have been reported by Gradojevitch (1939), and also by Hall (1950), and Wertheimer (1948). In the latter two cases the bony clavicular 'exotosis' was surgically removed.

An articular facet in the region of the conoid tubercle of the clavicle has long been recognized by anatomists as a rather uncommon osteological feature. Testut (1904) noted its occasional presence, Parsons (1916) found 4 in 286 bones, Jalůvka (1956) 25 in 491 clavicles, and Ray (1958) one pair in 292 Australian aboriginal clavicles. Isolated examples of coraco-clavicular joints have been recorded as rarities in dissecting room material by Bennett (1873), Gowland (1915), Meyer (1915) and Schlyvitch (1937).

In the dissecting room the author has frequently observed cartilage-covered articulating facets, of varying degrees of elaboration, on the clavicle and coracoid process together with an intervening synovial cavity even in the absence of any bony clavicular outgrowth. In this connexion it is of interest that Poirier (1890) considered that a true joint occurred in the region in three out of ten cases. Testut (1904) also, while recording three examples of the joint seen by himself, stated that joints here are far from being rare. These findings are clearly at variance with the generally accepted descriptions.

The present study was undertaken in an attempt to resolve the discrepancies in anatomical descriptions of this region.

MATERIAL

Forty-six arms from European dissecting-room subjects were examined. In some cases histological sections were prepared to confirm naked eye observations regarding the nature of the tissue covering articular facets. Several specimens were X-rayed and many dry bones were examined.

OBSERVATIONS

The attachments of the conoid and trapezoid ligaments

The attachments of these ligaments, where no bursae are present, are shown diagrammatically in Text-fig. 1. With the arm by the side the coracoid process points almost directly forwards and presents a superior surface with a tip and lateral and



Text-fig. 1. A diagrammatic representation of the arrangement and attachments of the left coraco-clavicular ligament. The trapezoid ridge, conoid tubercle, and groove for the subclavius are represented on the inferior surface of the clavicle. On the superior surface of the coracoid process the stippled and black areas represent the attachments of the trapezoid and conoid ligaments respectively.

medial borders. In its medial two-thirds the clavicle has descriptive anterior and posterior borders or surfaces. However, owing to the curvature of the bone, the anterior surface of the middle third looks laterally as well as forwards, i.e. the middle third of the bone approximates somewhat to the direction of the coracoid. The part

of the clavicle most intimately related to the upper surface of the coracoid process is at the lateral end of the groove for subclavius, and anterior to the conoid tubercle i.e. a little nearer the sternal end of the bone than the conoid tubercle itself. The part of the superior surface of the coracoid process immediately above the root forms a somewhat elevated and usually rather smooth area, limited laterally by a well-marked ridge which may form a somewhat overhanging lip separating it from the lateral surface of the root of the coracoid process. Medially this area is limited by another ridge which lies lower on the root posteriorly than anteriorly. The posterior part of the stippled area in Text-fig. 1 is often rather smooth or depressed and may be in either an almost vertical plane or in a more horizontal plane. The whole of the posterior area of the superior surface above the root, as just described, gives attachment to the coraco-clavicular ligament, the attachments of the conoid and trapezoid parts being shown in Text-fig. 1. On the clavicle the conoid and trapezoid parts of the ligament are attached respectively to conoid tubercle and trapezoid ridge. The conoid tubercle varies greatly in development. It may be no more than a slight roughening at the posterior border of the bone, appearing as a continuation of the trapezoid ridge. When the tubercle is large the conoid ligament, being a flat sheet, is attached only to its posterior part; the anterior part of the tubercle is then covered by an interlacing connective tissue mass made up of a continuation of the lateral end of the muscle subclavius with an admixture of fibres derived from the conoid ligament (Pl. 1, figs. 1, 2). The coraco-clavicular ligament as it passes between these coracoid and clavicular attachments is folded on itself as shown in Text-fig. 1. Occasionally a band of fibres derived from the conoid ligament sweeps across the lower (or lateral) aspect of the trapezoid ligament to be attached to the most lateral part of the trapezoid ridge. This band has been figured by Testut (1904).

The bursae

(1) *The bursa under the trapezoid ligament*

Sometimes the trapezoid ligament, instead of being attached to the whole of the stippled area on the coracoid shown in Text-fig. 1, may sweep across it to be attached to the more medial part only. The lateral part of the area is then cartilage-covered and separated from the under surface of the trapezoid ligament by a bursal cavity (Pl. 1, fig. 7). A lamina of the trapezoid ligament may also be attached to the lateral border of the area, so enclosing this bursa between the layers of the trapezoid ligament (Pl. 1, fig. 8). This bursa was present in eleven out of the forty-six specimens, though its size varied considerably.

(2) *The main bursa*

In the angle between conoid and trapezoid parts of the coraco-clavicular ligament is a quantity of fibro-fatty tissue frequently containing a large bursa. This is the bursa mentioned in most text-books (e.g. Johnston & Whillis, 1954). It intervenes between the clavicle above and the posterior part of the superior surface of the coracoid below, which is clothed by trapezoid ligament. The surface of the clavicle related to the bursa, and through it to that part of the coracoid clothed by trapezoid ligament, is at the lateral end of the groove for subclavius and just anterior to the conoid tubercle.

(3) *The conoid bursa*

When the most posterior part of the area on the coracoid for the attachment of the trapezoid ligament (that part above and lateral to the area for the conoid ligament) is more vertical than horizontal, the conoid ligament as it is folded forwards has its deep (or lateral) surface applied to this area, which of course is clothed by trapezoid ligament. Under these circumstances this investment of trapezoid ligament is converted into a fibro-cartilaginous facet separated from the deep surface of the conoid ligament by a definite bursal cavity (Pl. 1, figs. 1, 2, 5). This bursa may be quite independent of the main bursa previously mentioned, or may merely be an extension of it.

The coraco-clavicular joint

It has been noted that the main bursa intervenes between the coracoid where it is covered by trapezoid ligament, and the inferior surface of the clavicle at the lateral end of the groove for subclavius. These surfaces of the bones are frequently apposed and present well-marked cartilage covered articulating facets whose margins are connected by synovial membrane, constituting a true joint cavity (Pl. 1, figs. 1, 4, 8). Sometimes a facet is present on only one of the bones (Pl. 1, fig. 6). The articulating surface on the coracoid consists of a cartilaginous metaplasia of the trapezoid ligament where it clothes that bone; it varies in the perfection of its differentiation. Histological sections showed that the facet of the specimen illustrated in Pl. 1, fig. 4, consisted of well-differentiated fibro-cartilage; that shown in Pl. 1, fig. 1, was more highly elaborated and varied in structure from well-developed fibro-cartilage to hyaline cartilage. Cartilaginous change in the mass of tissue derived from the lateral end of subclavius, together with fibres derived from the conoid ligament, produces the articulating surface on the clavicle. To the naked eye the facet is smooth and cartilaginous—histologically it may vary from well-developed fibro-cartilage (Pl. 1, fig. 4) to quite well-differentiated hyaline cartilage (Pl. 1, fig. 1). These facets are normally in apposition and together with the synovial capsule uniting their margins (removed in the specimens illustrated in order to demonstrate the facets) constitute a gliding joint. In some cases the clavicle and coracoid are so separated that facets of this type would not be in contact. Thus, sometimes the clavicular articular surface surmounts a bony elevation, in the region of the attachment of the conoid ligament, which adjusts the clavicular to the coracoid facet. This produces an enlarged 'conoid tubercle' bearing an articular facet; the conoid ligament, of course, is attached only to its posterior border. This bony process is produced by ossification in the depths of a clavicular facet such as that shown in Pl. 1, figs. 1, 2. It is not strictly speaking a conoid tubercle and indeed, as would be expected, is often a little medial to this. A number of examples of clavicles bearing these articular processes have been seen in this study and Jalůvka (1956) & Parsons (1916) have analysed their frequency. Two examples are illustrated in Pl. 1, figs. 3, 10. One of these bones (Pl. 1, fig. 3) shows at its sternal end the typical coral-like texture and undulating grooves and depressions described by Todd & d'Errico (1928) as the typical appearance of the end of the growing shaft before union of the epiphysis. This epiphysis had either been detached, or more likely, had not commenced to ossify. The appearance fixes the age of the bone at under 21 years. Another similar

clavicle, also with a very well-developed articular process and facet has been seen in which the sternal epiphysis was just commencing to fuse again fixing its age at close to 21 years. A dissecting-room example of a coraco-clavicular joint complete with such an articular process on the clavicle, is shown in Pl. 1, fig. 8. It is seen that the muscle subclavius terminates against the articular process, and the conoid ligament is attached to its posterior margin. This is to be expected from the origin of the process by ossification in the depths of the clavicular facet. A small incomplete articular disc which is apparently fibrocartilaginous is present in this joint and both articular surfaces are covered by well-developed cartilage. Pl. 1, fig. 9 is an X-ray of this joint and shows the appearance of the clavicle which acts as the criterion by which clinicians have determined the presence of a joint. An articular disc, similar to that shown here, was present in another joint in this series, although in this case no clavicular process was present.

When the joint, a development of the main bursa, is present, the other bursae may also co-exist. Thus, in Pl. 1, fig. 8, a large and well-developed sub-trapezoid bursa is also present between the layers of the trapezoid ligament. In Pl. 1, fig. 1 a conoid bursa is also present, shown laid open in Pl. 1, fig. 2. In both these cases the additional bursae were independent. Thirteen joints of varying degrees of elaboration were found in this series, and of these one had a clavicular articular process.

One specimen has been seen of an articulation in another, though adjacent, situation. Here the articulation was more anterior, involving the clavicle close to its anterior border, and in front of the subclavius in its groove, and the coracoid process *in front* of the attachment of the coraco-clavicular ligament. This articulation was grossly pathological with worn and roughened articular surfaces and lined by synovial membrane bearing an exuberant mass of fringes and tags. Such an articulation clearly is of different nature to those described already.

DISCUSSION

The present study demonstrates that articulating cartilaginous or fibrocartilaginous facets on the clavicle and coracoid are common, though only in a few cases is there such extensive bony formation in the depths of the clavicular facet as to produce a marked bony process; a rudimentary elevation is shown in the case illustrated in Pl. 1, figs. 1, 2 and a well-marked process in Pl. 1, figs. 8, 9. Quite well-formed articulations may exist without the presence of this process, which merely adapts the articular surfaces to one another and is not a prerequisite for the presence of a facet. Studies of the frequency of coraco-clavicular joints based on X-rays (Moore & Renner, 1957; Nutter, 1941; Wertheimer, 1948) are inadequate, for they merely show the incidence of these clavicular processes, those joints without them possessing no distinguishing roentgenographic feature. Similarly, examination of dry bones only shows the frequency of these processes (Parsons, 1916; Jalůvka, 1956) and not of joints.

There is a gradual transition seen between a bursa and the presence of a well-developed articulation. The point at which the arrangements warrant description as a joint will remain a matter of opinion, but a true joint is certainly quite common.

The findings here agree with the statement of Poirier (1890) that a joint exists in three out of ten cases. Schlyvitch (1937), as the result of a dissecting-room survey of this joint, states that in only one of sixty cadavers was a true diarthrosis present, but it appears that his criteria were unusual, for he adds that six others had apposing cartilage-covered surfaces (and eight others had contacting bony surfaces covered by fibrous tissue). Thus, it appears that it is his interpretation and not the results which are in conflict with the present findings. Thus, the coraco-clavicular joint should not be regarded as an anomaly but as a common feature varying in the degree of its elaboration. The joint will presumably be subject to pathological change, but X-rays give no sure guide to its presence.

Lane, Poland & Dunn (1887), reporting on dissecting-room findings, stated 'in many instances we saw the coraco-clavicular joint well-developed, the subclavius being inserted into the capsule of that articulation' (i.e. a joint in the position as described in this paper). However, in 1886 Lane had described the joint as being more anterior, between the under surface of the clavicle *in front* of the subclavius, and the coracoid between the attachment of the coraco-clavicular ligament and the tip. In 1888 Lane distinguished two joints: an anterior one, which he considered to occur frequently in various labourers, and corresponding in the present series to the single pathological example mentioned here; and a posterior one, corresponding in position to those described in this study, but which he considered to occur only in the shoe-maker, being related to the particular movements associated with his work. However, there is no doubt from the present findings, that the anterior form is rare, if ever normally present. It is the posterior variety which is the common type.

It is well known that the development of any structure in an animal is dependent upon the interaction of internal and external factors (De Beer, 1951). Any tissue has an inherent power of response, genetically controlled and inherited, which may be called forth by changes in the environment or use. It is known that localized pressure and friction may induce metaplasia of ordinary connective tissue into cartilage (Le Gros Clark, 1952; Maximow & Bloom, 1957). Also, connective tissue, under certain circumstances, can produce synovial-lined cavities and such new bursae have been produced experimentally (Wilson, 1948). Indeed, complete new joints, pseudarthroses, may arise at the site of an ununited fracture. Therefore, the capacity to form new joints under changed conditions is normally possessed. For instance, a joint is normally present between the acromion and upper anterior part of the head of the humerus, in the armadillo (Flower, 1885). Similarly, the coraco-clavicular joint may arise due to new conditions of use, and indeed is said to be a regular finding in the gorilla and gibbon (Köhler, 1935).

Two young clavicles (under 25 and probably less than 21 years) showed clear evidence of the coraco-clavicular joint in its most highly elaborated form. The smoothly moulded contours of these bones, with their well-developed articular processes for the coracoid, make it appear most likely that the joints were present at birth. It therefore appears probable that sometimes at least the coraco-clavicular joint is an inherited character present before birth, and so before conditions of use alone could determine its formation.

SUMMARY

1. The attachment of the conoid and trapezoid ligaments, and the bursae related to them, are described in detail.

2. Cartilage-covered articular facets of the clavicle and coracoid process, separated by a synovial cavity and forming a true synovial joint, were found to occur quite frequently.

3. The absence of an articular process on the clavicle in the region of the conoid tubercle does not exclude the presence of a well-marked articular facet or coraco-clavicular joint.

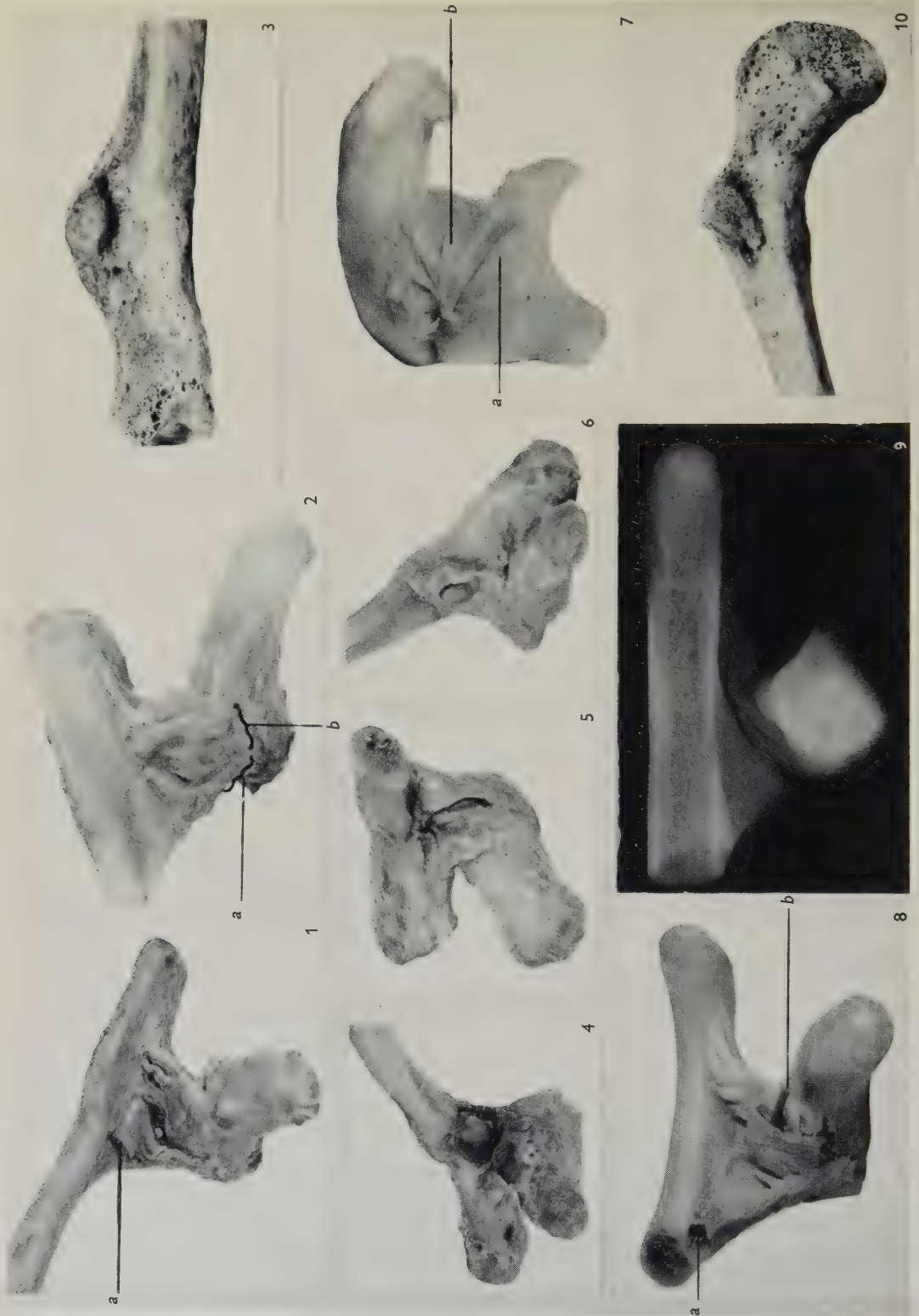
4. Estimates of the frequency of coraco-clavicular articulations, based on the presence of this process observed radiologically or in osteological material, are misleading.

5. Well-marked articular processes were observed on two immature clavicles. The possible significance of this is briefly considered.

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EXPLANATION OF PLATE

- Fig. 1. A well-developed left coraco-clavicular joint. The synovial membrane which enclosed the joint cavity is removed to demonstrate the plane articular facets which are separated by elevation of the sternal end of the clavicle. The formation of the facet on the clavicle from the lateral end of the subclavius (*a*) and from some fibres of the conoid ligament can be seen, while the coracoid facet consists of cartilaginous change in the trapezoid ligament. A conoid bursa is also present in this specimen deep to the conoid ligament.
- Fig. 2. The same specimen as shown in fig. 1. The conoid ligament has been severed from its lower attachment (*b*) and the cut surface is shown (*a*). Thus, the conoid bursa is opened; the smooth cartilaginous surface on the coracoid against which the deep surface of the conoid ligament glides is shown just above this ligament's attachment (*b*). The anterior part of the trapezoid ligament, and thus of the coracoid facet, is removed. The greater part of the clavicular facet is removed, leaving only that part derived from conoid ligament, and showing the low bony elevation which was present in its depths.
- Fig. 3. A left clavicle with a well-developed articular process.
- Fig. 4. A rather simple right coraco-clavicular articulation. The sternal end of the clavicle is lifted to separate the articular facets which are normally in apposition, and the synovial membrane enclosing the joint cavity is cut open anteriorly.
- Fig. 5. Right coracoid and clavicle with coraco-clavicular ligament, showing a conoid bursa deep to the conoid ligament and opened anteriorly.
- Fig. 6. Left coracoid and clavicle with coraco-clavicular ligament. A main bursal cavity is opened to show a single clavicular facet at the lateral end of the groove for subclavius.
- Fig. 7. Right coracoid and clavicle viewed laterally. A trapezoid bursa intervenes between the smooth cartilage-covered lateral part of the upper surface of the coracoid (*a*) and the trapezoid ligament. The synovial membrane which formed the lateral wall of the bursa has been cut and lifted (*b*).
- Fig. 8. A well-developed left coraco-clavicular joint. There is a well-developed clavicular articular process in this specimen against which the subclavius (*a*) terminates laterally. The anterior capsule of the joint is removed showing the plane articular surfaces, separated by elevation of the clavicle, and the incomplete articular disc between them. The pointer (*b*) is inserted into a fairly extensive trapezoid bursa situated between the layers of the trapezoid ligament.
- Fig. 9. Radiograph of the specimen shown in fig. 8 illustrating the clavicular process.
- Fig. 10. A right clavicle showing a well-developed articular process.

THE INFERIOR MESENTERIC GANGLION

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The existence of an inferior mesenteric ganglion in man is either not mentioned or is disputed in some standard anatomical text-books, and apparently very few workers have attempted to prove or disprove its presence by macroscopic and microscopic studies. It is shown by Schäfer & Thane (1895) in a diagram in the 10th edition of Quain's *Elements of Anatomy*, but is not described in the text. Petit-Dutaillis & Flandrin (1923) found a single ganglion on the left side of the inferior mesenteric artery in five out of thirteen subjects; Hovelacque (1927) reported an inferior mesenteric ganglion in 50 % of cases, and in these it was always placed to the right of the median plane. Kuntz (1940) described the histology of such a ganglion. Mitchell (1953) stated that a ganglion may exist just above the origin of the inferior mesenteric artery, or more often minute discrete ganglia are found both above and below the root of the artery or scattered in the proximal part of the plexus. Kuntz & Jacob (1955) described minute ganglia in the inferior mesenteric plexus. Clearly there is no unanimity about the ganglion or ganglia so it was decided to re-investigate the problem.

MATERIAL AND METHODS

Twenty-two stillborn infants, ranging in age from 7½ to 9 months, were dissected after preliminary injection of 100–150 ml. of embalming fluid via the umbilical vein. To facilitate access the liver, spleen, stomach and intestines proximal to the mid-part of the transverse colon were removed. The pelvis was split through the pubic symphysis and the halves distracted. The inferior mesenteric region was then dissected carefully under a 30 % alcohol–water mixture with the aid of a dissecting lens. The specimen was then immersed in 20 % formalin for 3 days in order to harden and bleach the nerves.

OBSERVATIONS

In each infant dissected an inferior mesenteric ganglion (or ganglia) was found to be present. It was not easy to isolate, being embedded in a meshwork of fibrous tissue and the nerves of the inferior mesenteric plexus; many of these nerves had to be divided in order to expose it clearly. The para-aortic bodies in this region were easily differentiated from the ganglion owing to their reddish brown colour and friability; the ganglion itself was tougher in consistency. Care was taken not to confuse the inferior mesenteric ganglion with the ganglia of the intermesenteric nerves.

POSITION AND APPEARANCE

The position of the ganglion is not entirely constant, but there are two typical sites. In about a third (8/22) of the dissections the ganglion lay directly anterior to the root of the inferior mesenteric artery (Fig. 1). In another third (7/22) it lay on the left side in the angle between the inferior mesenteric artery and its upper left colic

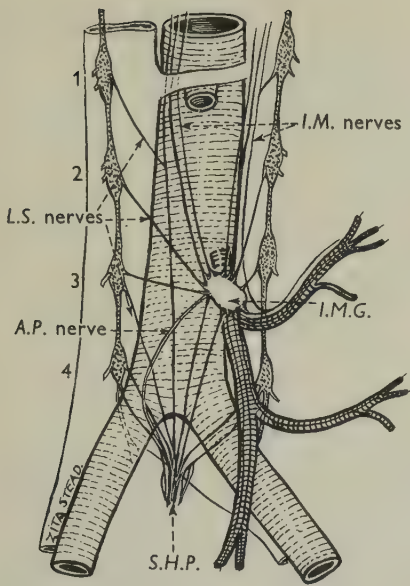


Fig. 1

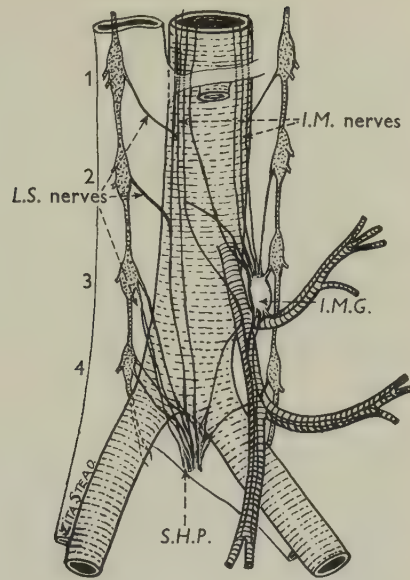


Fig. 2

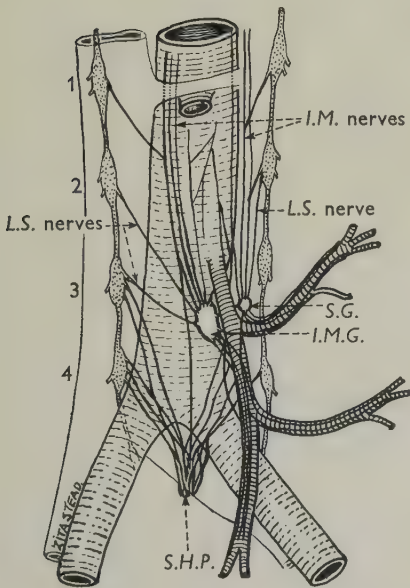


Fig. 3

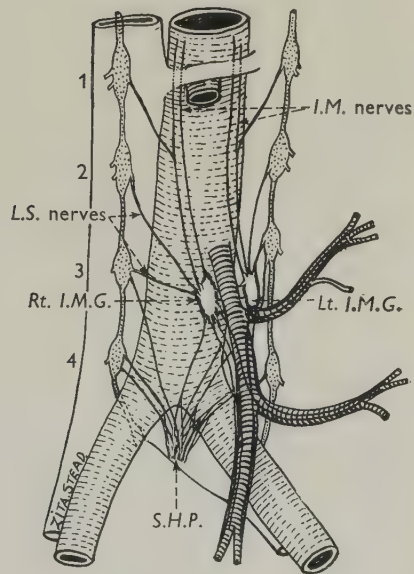


Fig. 4

Figs. 1-4. *I.M. nerves*, intermesenteric nerves; *I.M.G.*, inferior mesenteric ganglion (*Rt.*, right); (*Lt.*, left); *L.S. nerves*, lumbar sympathetic nerves; *S.H.P.*, superior hypogastric plexus; *A.P. nerves*, ascending parasympathetic nerves; *S.G.*, small ganglion.

branch (Fig. 2). In four other dissections the ganglion lay on the aorta along the right border of the inferior mesenteric artery (Fig. 3), and in these minute ganglia were also present in the inferior mesenteric plexus near its origin. In the remaining three specimens two ganglia were observed, one on either side of the artery (Fig. 4).

The majority of these ganglia are situated about a quarter of an inch below the origin of the inferior mesenteric artery from the aorta. Two, however, were half an inch below and one was directly anterior to its origin.

In every specimen the ganglion was large enough to be seen with the naked eye. Eleven were approximately 1 mm. in diameter, seven measured about 2×1 mm., and four were smaller, being about $\frac{1}{2}$ mm. in diameter.

The shape of the ganglia was variable, but most were either stellate or oval with the long axis in the line of the artery.

CONNEXIONS

Three or four nerves from the lumbar sympathetic trunks of each side pass either to the intermesenteric nerves or to the ganglion itself. Each first lumbar ganglion contributes a single filament to the intermesenteric nerves. Two or three filaments from the second and sometimes from the third lumbar ganglia pass directly to the upper pole of the inferior mesenteric ganglion, or to the immediately adjacent parts of the intermesenteric nerves. Four to eight intermesenteric nerves pass down each side of the aorta, usually in two bundles. More of these fibres descend on the left and often lie a short distance lateral to the aorta. The right intermesenteric nerves terminate in the right upper pole of the ganglion and the superior hypogastric plexus. The nerves on the left side end in the ganglion and superior hypogastric plexus, or join the nerves accompanying the upper left colic artery.

The inferior mesenteric plexus, consisting initially of six or more interconnected bundles, arises from the lower pole of the ganglion and passes along the inferior mesenteric artery. The bundles subdivide and follow the arterial arcades and their terminal filaments enter the medial surface of the descending colon and the mesenteric border of the sigmoid colon. One or two thicker bundles descend from the postero-inferior surface of the ganglion to the superior hypogastric plexus.

Nerves ascending from the left hypogastric plexus to the inferior mesenteric plexus, described by Telford & Stopford in 1934, were seen in half the specimens. In three, however, a nerve was observed ascending from the right hypogastric plexus to the lower pole of the ganglion (Fig. 1), and in another specimen a similar nerve was not connected with the ganglion but joined the nerves alongside the superior left colic artery near its origin.

Where two ganglia were present, the lumbar splanchnic and intermesenteric nerves joined the ganglion of their own side and these ganglia were interconnected by several fascicles passing behind the origin of the inferior mesenteric artery. From the lower pole of each ganglion nerves passed alongside the artery and branched to form a plexus around it. Most of the nerves from the left ganglion passed to the left colic branches, while those from the right ganglion continued into the pelvic mesocolon with the parent artery and its branches (Fig. 4).

HISTOLOGY

The ganglionic and nervous nature of the structures described was confirmed in seven typical specimens by microscopic examination.* Serial sections were prepared and stained with either haematoxylin and eosin, cresyl violet or silver. All showed the presence of sympathetic ganglionic neurons, a number of which were binucleated.

The vascularity of the substance of the ganglia was notable, a finding which has been reported from studies of other sympathetic ganglia (Patterson, 1950).

DISCUSSION

Brown & Pascoe (1952) noted that the inferior mesenteric ganglion in the rabbit is a single unpaired structure lying in the angle between the inferior mesenteric artery and the aorta. This is similar in position to a number of those found in the human foetus, although they were generally situated rather more distally to the origin of the artery. Harris (1943) described in the cat three discrete ganglia in a triangle around the inferior mesenteric artery. In human material Kuntz & Jacob (1955) invariably found microscopic ganglia along the inferior mesenteric artery and its branches, but few were large enough to be detected macroscopically.

In every infant of the twenty-two dissected in the series recorded here one or two macroscopic ganglia were visible. Perhaps the reason why they have not been found constantly by previous workers is because they are usually so firmly embedded in the nerves of the inferior mesenteric plexus. These nerves have to be teased away individually with the aid of fine dissecting instruments until a solid object, often in the centre, is exposed. The ganglion can then be recognized as a small oval body with numerous nerve connexions.

As well-defined ganglia are situated in relation to the two other unpaired visceral branches of the abdominal aorta, it is not surprising that one or two macroscopic ganglia should also be found adjacent to the third branch, the inferior mesenteric artery; in addition, microscopic ganglia lie more distally along its branches.

The nerve found ascending on the right side from the superior hypogastric plexus to the ganglion may carry parasympathetic fibres to the distal colon, just as those found on the left side described by Telford & Stopford (1934).

SUMMARY

1. Twenty-two human stillborn infants were dissected to investigate the existence of the inferior mesenteric ganglion (ganglia).

2. Macroscopic ganglia were found in each case. In eight specimens a single ganglion lay anterior to the inferior mesenteric artery, in seven along its left border, and in four along its right border. In the remaining three dissections two well-defined ganglia were isolated.

3. The ganglia were oval or stellate in shape and varied in size between 0.5 and 2 mm.

4. The connexions of the ganglia are described, including one nerve ascending to it on the right from the superior hypogastric plexus.

5. The presence of ganglionic neurons was confirmed by microscopic examination.

* Owing to the cost of publication it has been necessary to omit photographs of the dissections and histological preparations.

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POSTNATAL CHANGES IN THE SHAFT OF THE RAT'S FEMUR

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INTRODUCTION

The development of our knowledge of bone growth and that of bone structure have gone along distinct paths, and there has been little synthesis between the two approaches. The following account attempts such a synthesis and describes the changes in structures in a rat's femur which occur during the bone's growing period, and as a result some new concepts are presented.

The growth cartilages of the long bones of most mammals disappear early in life and this feature is taken as an indication of the maturity of the individual, but in the rat there is a persistence of the growth cartilages into adult life (Dawson 1925, 1929). However, the rat is not really so different as might appear on first sight, as there is a prior cessation of longitudinal bone growth (Becks, Simpson & Evans, 1945). Also, the long bones of the rat do not contain Haversian systems such as are found in larger and longer-living mammals but, as Ruth (1953) has shown, the formation of such structures can be induced in the rat, and this led him to believe that the normal development of Haversian systems was fortuitous and was essentially a repair process.

Thus, if these structural differences reported in the bones of the rat are of no great significance, then the findings of the present account may well have a general application amongst the mammals. It would appear that there is a marked similarity in the pattern of growth as seen in the corresponding bones of all the mammals so far investigated experimentally. There is a lack of precise knowledge about the growth and structure of human bones, but, as will be pointed out in the discussion, there are certain apparently obscure disorders of human bone growth which can be interpreted in the light of the present findings.

METHODS

The account is based on the femurs taken from twenty-six rats of both sexes which were killed at intervals between birth and 322 days. In each case the bone was fixed in 5% formol-saline, and decalcified in a 0.5 M. solution of the disodium salt of ethylene-diamine-tetra-acetic acid. Sections were cut at $4\ \mu$ after embedding in paraffin wax. Some sections were impregnated with silver, using an abbreviated Long's method, as an alternative to the usual staining methods.

OBSERVATIONS

(a) *Neonatal period (1-9 days)*

The pattern of growth that occurs during the neonatal period is a continuation of that seen during the late foetal period (Pratt, 1957). The neonatal femur consists of a cylinder of periosteal bone which lengthens at its extremities, and at the same time increases in width throughout its length (Text-fig. 2a). This periosteal bone is cancellous, with irregularly arranged and shaped lacunae, and the fibres of its matrix form a meshwork of coarse and fine bundles. The trabeculae forming the extremities of the periosteal cylinder are linear and contain thick longitudinally directed fibres which have been secondarily incorporated into the bone, and which later, due to the elongation of the bone, are found also in the middle portion of the shaft.

The perichondrial collar of bone, whose matrix contains short radially directed fibres, lines the periosteal cylinder and separates this from the endochondral bone at the metaphyses. The collar consists of two truncated cones, lying apex to apex, the diverging extremity of each cone ensleeving the cartilagenous end of the diaphysis (Text-fig. 1a). The central part of the collar is lost during the process of intramedullary erosion, the latter being particularly active about the 2nd day at which time the marrow cavity loses its hour-glass outline and becomes tubular. The external surface of this bone is continuous with the matrix of the periosteal bone, though the latter never extends beyond the level of the invasion front. The collar is lined on its deep surface by a layer of apparently fibreless cartilage matrix, upon which endochondral bone is deposited.

The metaphysis may be defined as a core of secondary spongiosa which is surrounded by perichondrial bone and is in turn inserted into the extremity of the diaphysial tube. The deposition of endochondral bone is confined to the metaphysis. The matrix of this bone is distinctive and contains fine fibre bundles which form an irregular network.

(b) *Changes in the metaphysis associated with the appearance of endochondral ossification in the distal epiphysis (11-14 days)*

Endochondral bone appears in the distal epiphysis on the 11th day. However, before this happens the cells of the epiphysial cartilage hypertrophy, and as a result there is an increase in size of both the epiphysial and growth cartilages. This effect occurs rapidly, that is over the period of a few days, and the distal end of the bone takes on a clubbed appearance (Text-figs. 1b, c).

The large metaphysis consists almost entirely of perichondrial and endochondral bone (Text-fig. 1c). The perichondrial bone which ensleeves the metaphysis soon becomes perforated, this process commencing on the deep surface where there are large numbers of osteoclasts. The sites of interruption are plugged with undifferentiated connective tissue cells derived from the subperiosteal space.

The bone which forms the diaphysis is similar in structure to that of the neonatal shaft, that is, it is cancellous and its matrix contains both fine and coarse fibre bundles which are irregularly interwoven. There are, however, no needle-like trabeculae at the distal end of the diaphysis, and subperiosteal osteogenesis does not extend



Text-fig. 1. Early postnatal changes in the distal femoral metaphysis of the rat ($\times 22.5$). (a) 1 day. The metaphysis is completely overlapped by the periosteal cylinder. (b) 7 days. The metaphysis is commencing to separate from the periosteal cylinder. (c) 14 days. The metaphysis is clubbed and unsupported by periosteal bone. A secondary centre has appeared. (d) 21 days. The metaphysis is becoming incorporated into the shaft. Cartilage stippled. Endochondral endosteal bone hatched. Perichondrial bone shown as brush border. Periosteal bone black.

on to the perichondrial bone of the metaphysis. The coarse longitudinally directed extraosseous fibre bundles, which were seen during the neonatal period, and which passed into the bone of the extremity of the diaphysis, now form a thick sheath which surrounds the perichondrial bone of the metaphysis.

The most lateral of the trabeculae of spongiosa are not removed during medullary erosion. Thus the outline of the distal extremity of the medullary cavity is domed, and the perichondrial bone is buttressed on its internal surface by these persisting trabeculae of endochondral bone. This latter feature will compensate for the lack of periosteal bone supporting the external surface.

(c) *The incorporation of the distal metaphysis into the shaft (17 days onwards)*

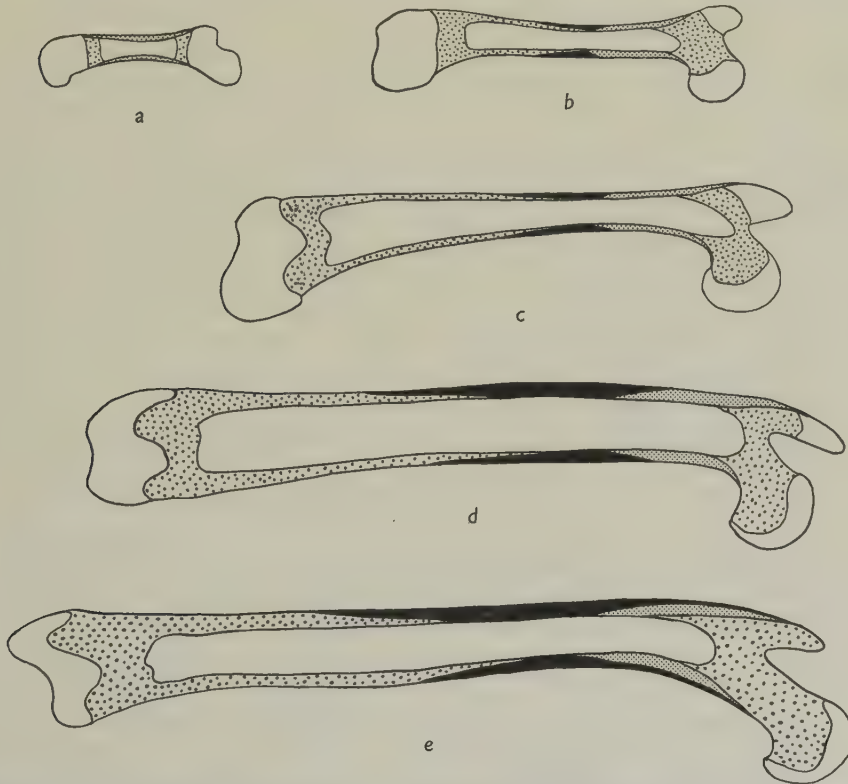
The bone is beginning to lose its clubbed appearance by the 17th day (Text-fig. 2*b*). This is due to the disappearance of the perichondrial bone which ensleeved the distal metaphysis, and to the resorption of the adjacent secondary spongiosa. At this time there are large numbers of osteoclasts on the external surface of the metaphysis.

Remains of the original cone of perichondrial bone do persist in certain places. The more central portion separates the periosteal bone from the endochondral bone, and its hyaline internal border appears as a diagonal band crossing the shaft. However, it has been removed by medullary erosion by the 21st day, and in its place there is a cement line which also separates the periosteal bone from the endochondral bone (Text-fig. 3*e*). The perichondrial bone continues to be formed adjacent to the growth cartilage, only to be removed by the process of metaphysial remodelling. The fibres of this bone are longer and present a more shaggy appearance as compared with the fibres seen in the earlier perichondrial bone. This sole remaining portion of the perichondrial collar has been called the perichondrial ring of the ossification groove (Lacroix, 1945). Its formation ceases posteriorly at 38 days and is absent elsewhere shortly afterwards.

The remodelling of the metaphysis allows a portion of this segment to become absorbed into the shaft (Text-figs. 2*b* and 1*d*). This 'incorporated metaphysis' is characterized by a lack of subperiosteal osteogenesis. The way in which this segment of the shaft becomes consolidated requires comment. From the 14th day endochondral bone is laid down in the metaphysis in greater amounts, especially on the more peripheral cartilagenous trabeculae. As the metaphysis becomes incorporated into the shaft further bone is laid down on the already reinforced spongiosa. This former bone is sometimes referred to as endosteal bone, and it is useful to retain this term if certain objections to it can be reconciled. First, it is difficult to be certain as to the origin of the osteoblasts which form this bone. They may be derived from the undifferentiated subperiosteal cells which extend between the peripheral trabeculae of the metaphysis or, alternatively, they may be derived from the same source as the osteoblasts which form the endochondral bone. This latter possibility leads on to the second argument against the conception of endosteal osteogenesis as an entity, that is the fibrous structure of endochondral and endosteal bone may be indistinguishable.

The fibrous structure of the metaphysial bone is complex (Text-fig. 3*d* and Pl. 2, fig. 9). The endochondral bone now contains fine plaited fibre bundles which are

irregularly arranged, and this is the only bone matrix seen adjacent to the growth cartilage. In some situations the endosteal consolidation appears to be a continuation of endochondral deposition, the only distinction between the two being that the endochondral bone is a thin layer with only occasional lacunae, while the endosteal bone is a thick layer with numerous lacunae. However, elsewhere there may be a cessation of endochondral deposition of bone, and a cement line separates this bone from the endosteal bone that is laid down later. Here the plaited fibre bundles of the endosteal bone tend to be arranged parallel to each other. A similar structure



Text-fig. 2. The postnatal development of the shaft of the rat's femur. ($\times 4$.) (a) 4 days. The shaft consists of a cylinder of periosteal bone ('primitive diaphysis'). (b) 17 days. The distal metaphysis is being incorporated into the shaft and is overlapped by the 'splinting diaphysis'. The proximal portion of the shaft is formed by the 'primitive diaphysis'. (c) 38 days. The 'incorporated metaphysis' forms a major portion of the shaft. (d) 84 days. The 'splinting diaphysis' becomes more extensive. (e) 322 days. The 'primitive diaphysis' persists into the adult. Metaphysis and 'incorporated metaphysis'—coarse stipple. 'Splinting diaphysis'—black. 'Primitive diaphysis'—fine stipple.

is found in the endosteal bone, which gives rise to the internal buttressing about the level of the overlap of the periosteal bone. Here the fibre bundles are arranged in rows, are orientated longitudinally, and the lacunae are elongated with their greatest diameter being in the long axis of the bone.

It will be recalled that the junction of the metaphysis and diaphysis at 14 days

was supported by a short overlap of periosteal bone (Text-fig. 1c). This bone continues to form as the metaphysis becomes incorporated into the shaft and remains separated from the former by a cement line. This cement line forms on the 'incorporated metaphysis' in the absence of subperiosteal bone deposition. The periosteal bone concerned in the overlapping of the 'incorporated metaphysis' will be referred to as the 'splinting diaphysis'. It has a characteristic fibre structure consisting of plaited bundles arranged in rows and lying in a longitudinal direction. The lacunae are elongated with their greatest diameter lying in the long axis of the bone. It should be pointed out that, in the course of time, the 'splinting diaphysis' comes to form a full-thickness portion of the shaft, due to the internal erosion of the 'incorporated metaphysis' (Text-figs. 2b-e).

The more proximal portion of the diaphysis continues to form in the same manner as it did during the neonatal period. That is, it remains trabecular, though the vascular spaces become reduced in size with increasing age. Its trabecular structure is explained by the continued incorporation of coarsely bundled extraosseous fibres, as these fibres are continuing to form at the proximal end of the femur where no metaphysial incorporation is occurring. The osseous fibre bundles are irregularly arranged, thus giving a woven appearance to the bone, though the finer fibres are plaited in a way similar to the fibre bundles found elsewhere at this stage. Because of its distinctive fibrous structure, this part of the diaphysis will be referred to as the 'primitive diaphysis'. It should be pointed out that the boundary between the 'splinting diaphysis' and the 'primitive diaphysis' is ill-defined, and both are constantly eroded on their medullary surfaces, where numerous osteoclasts may be seen.

(d) *Changes in the shaft and distal metaphysis associated with the slowing of the growth of the bone. (84 days onwards)*

Text-fig. 2 shows that the 'incorporated metaphysis' and the 'splinting diaphysis' together form the major portion of the definitive shaft, and the 'primitive diaphysis' appears to remain static in regard to its contribution to the length of the shaft.

Lamellar bone appears at 84 days and its deposition is confined to the periosteal and medullary surfaces of the overlap area. The lamellae are arranged parallel to the surface. The fibres of this bone form layers of longitudinally running bundles, each layer of which is separated by a layer consisting largely of radially directed fibres, but containing a variable amount of circumferential fibres, thus giving the appearance of feathering on longitudinal sections if the latter fibres are few in number (as in Pl. 2, fig. 6). The lacunae of this bone are sparsely distributed and very elongated. The transition from the parallel bundles of plaited fibres to the feathered lamellar bone is gradual. There is an increasing separation of the plaited bundles, the linking fibres between adjacent bundles come to form the layer of radial fibres of the lamellar bone.

Metaphysial remodelling appears to have ceased by 157 days, for there is no surface osteoclasts. This is no doubt associated with the greatly diminished activity of the growth cartilage, with the consequent stability in the diameter of the metaphysis. This slowing of growth allows the 'endosteal' consolidation of the secondary spongiosa to extend farther into the metaphysis, and for the first time since the neonatal period, subperiosteal osteogenesis is occurring in the metaphysis. As this

bone formation is merely an extension of endochondral osteogenesis, no cement lines are present. It must be pointed out that this subperiosteal osteogenesis is confined to the metaphysis and does not extend into the 'incorporated metaphysis'.

At 190 days, lamellar bone appears within the distal metaphysis (Text-fig. 3a), where it consolidates trabeculae and fills up vascular spaces. There is no evidence that this bone is in any sense a replacement bone as it is frequently a continuation of the earlier endosteal deposition, and elsewhere, where cement lines separate this lamellar bone from the earlier endosteal bone, there are always resting lines. The fibrous structure of this lamellar bone (as in Pl. 2, fig. 7) is similar to that seen at 84 days.

The process of medullary erosion within the shaft appears to slow down after 84 days, for after this oestoclasts are rarely seen on its internal surface. Nevertheless, at 322 days the subperiosteal deposition of lamellar bone is continuing in the 'splinting diaphysis' and endosteal lamellar bone is being deposited about the overlap area of the 'incorporated diaphysis' (Text-fig. 3b). This lamellar bone has a different structure, as the feathered appearance of the earlier lamellar bone is only rarely found, and the bone now consists of alternate layers of longitudinal and circumferential fibres (Pl. 2, fig. 8).

The primitive diaphysis retains its irregular and heterogeneous fibrous structure throughout the growing period of the bone (Pl. 1, figs. 1-3). It becomes more compact, but even at 322 days in longitudinal sections there can be seen numerous obliquely running vascular channels. The subperiosteal deposition of lamellar bone gradually encroaches upon this segment of the shaft, until at 322 days the 'primitive diaphysis' is completely ensleeved by lamellar bone (Text-fig. 3c). The transition from woven bone to lamellar bone is shown in Pl. 1, figs. 3-5. It is characterized by a disappearance of the obliquely running coarse fibre bundles and by an intermediate stage, where the fine fibres become collected into groups of bundles which are orientated both longitudinally and circumferentially.

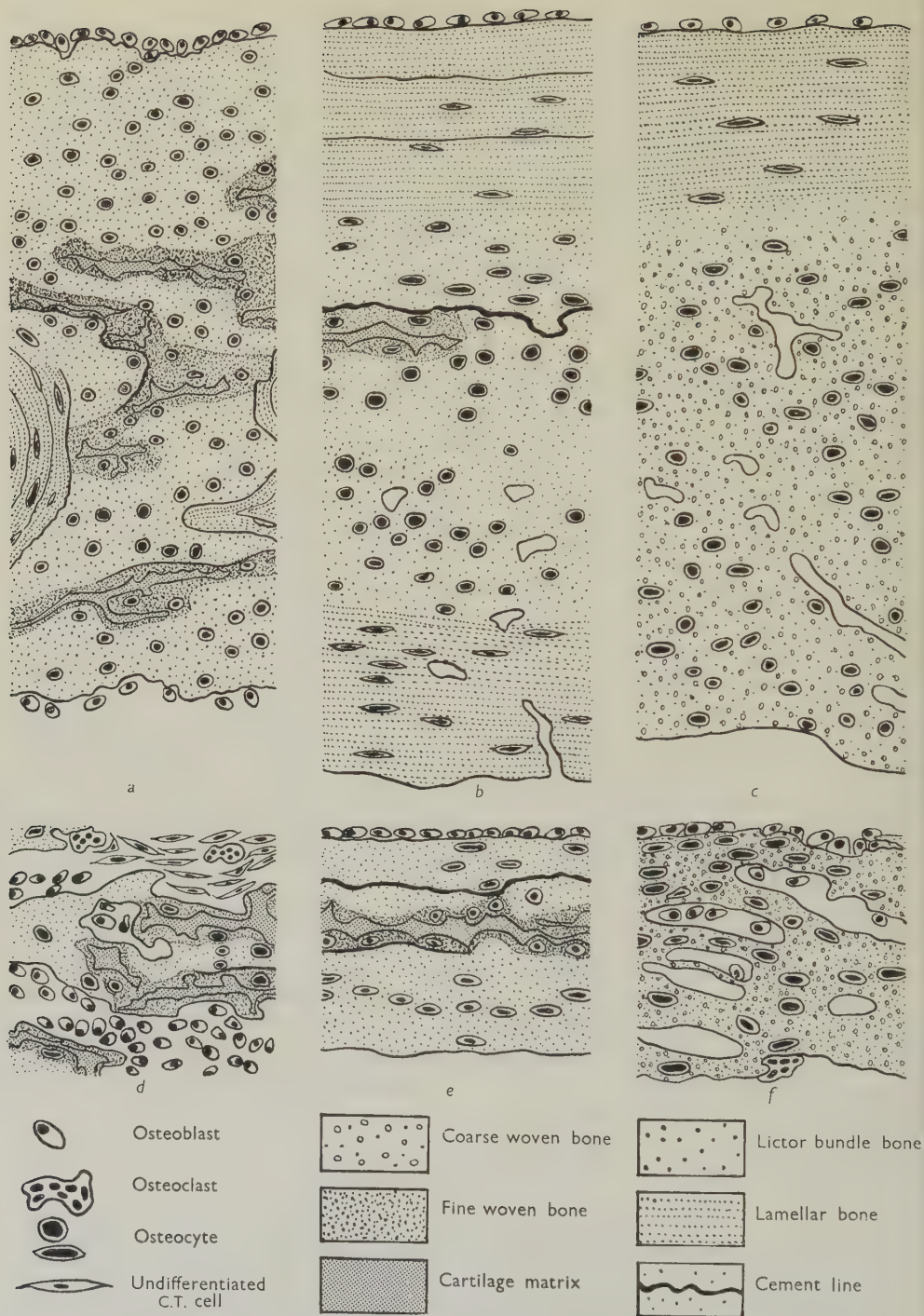
The proximal metaphysis

The proximal growth cartilage grows more slowly than the distal cartilage, consequently the former contributes little to the elongation of the bone, and is not incorporated into the shaft (Text-fig. 2). This no doubt accounts for the persistence of the primitive diaphysis as an entity throughout the growth period. The histological changes are otherwise similar in both metaphyses.

DISCUSSION

(1) *Neonatal pattern of growth*

It is often stated or implied that a mammalian long bone changes its form solely by the elongation and widening of its periosteal cylinder. The present account shows that in the femur of the rat such a pattern of growth occurs and is found in early postnatal life, but is soon replaced by a more complex pattern. This is in agreement with the findings in the autoradiographic experiments with the neonatal rat of Greulich & Leblond (1953), and those of Leblond, Wilkinson, Bélanger & Robichon



Text-fig. 3. The distribution of the different types of bone matrix as seen in longitudinal sections at 322 days (a-c), 17 days (d-f). Periosteal surface uppermost ($\times 200$). (a, d) Metaphysis. (b, e) Junction of 'incorporated metaphysis' and 'splinting diaphysis'. (c, f) Primitive diaphysis.

(1905). Bhaskar, Weinmann, Schour & Greep (1950) showed a similar simple pattern of growth in the tibia of the neonatal rat.

It is probable therefore that those disorders of human bone growth, which are found at or about the time of birth, may well be very different from those found later. In the same way a pathological lesion of a bone may well present very different appearances according to the current pattern of growth.

(2) *The normal clubbing of the metaphysis*

Bhaskar, Weinmann and Schour (1954) showed that there was, immediately prior to the appearance of a secondary centre, an increase in all the dimensions of the proximal end of the rat's tibia. The clubbed appearance of the distal femoral metaphysis of the rat at 14 days also follows the appearance of a secondary centre and this is doubtless associated with the changes in growth similar to those described by Bhaskar *et al.* (1954). It would appear that the morphological and histological features of metaphysical clubbing have escaped recognition in the past, and this is no doubt due to the short duration of this phase as remodelling soon commences and the clubbing disappears.

There are numerous records of pathological clubbing of the metaphysis, but there has been no attempt in the past to correlate this with the normal growth process. The grey-lethal mouse (Grüneberg, 1935; Bateman, 1954) and the microphthalmic mouse (Grüneberg 1948; Bateman, 1954) both show complete arrest of growth at about 15 days and clubbing of certain metaphyses. The latter is explicable if there was an arrest of all growth of the bone at the clubbing stage, and it is not necessary to implicate the failure of the osteoclastic activity, though this may be the case elsewhere in the skeleton. In hereditary osteopetrosis of the rabbit (Pearce, 1948, 1950) there is clearly an arrest of growth at the normal clubbing stage but, in those individuals which survive for any length of time, the appearances change, due to the continued periosteal deposition of bone.

It is suggested therefore that a clubbed metaphysis is a stage in normal bone growth and is due to the rapid rate of growth of the epiphysial cartilage at the time of the appearance of the secondary centre, while at the same time the periosteal deposition of bone appears to lag behind. The clubbing is transitory and easily overlooked, but may become apparent when growth is arrested at this stage. When this occurs the condition must be distinguished from the failure of remodelling of the 'incorporated metaphysis' (which will be discussed in more detail later). The normal clubbing process results in a flask-shaped extremity to the bone, while the failure of remodelling of the 'incorporated metaphysis' gives the extremity a bottle-shaped appearance.

(3) *The 'incorporated metaphysis'*

Brash (1934) and Payton (1932), using the pig, repeated the madder experiments of earlier workers but did not comment upon the growth patterns. However, the illustrations of the latter worker showed, at either extremity of the shaft, a truncated cone of uncoloured bone, the base of which was adjacent to the metaphysis. It is probable that this uncoloured zone contained bone which was deposited in the metaphysis before the madder was given and corresponds, in the rat, to the

endochondral and endosteal bone which lies immediately adjacent and internal to the oblique cement line. Aries (1941), after having administered alizarin to rats, described the pattern of growth in the femur and showed that the bone elongated by the 'superimposition of serial cones within the diaphysial portion of the shaft, the bases of which cones are the epiphysial plates at any given period.' He also showed that the periosteal depositions of bone did not contribute towards the longitudinal growth. The former finding was confirmed by the autoradiographic experiments of Leblond *et al.* (1950), which showed that the shaft of the rat's tibia consisted of a central cylinder and two peripheral funnels, and that the latter were being constantly transformed into the former. The junction of the funnel and the cylinder was either described or illustrated by several later workers, who also used autoradiographic methods in a variety of mammals (Comar, Lotz & Boyd, 1952; Kidman, Rayner, Tutt & Vaughan, 1952; Jowsey, Owen & Vaughan, 1953; Jowsey, Rayner, Tutt & Vaughan, 1953; Tomlin, Henry & Kon, 1953; and Owen, Jowsey & Vaughan, 1955). However, in no instance was the significance of the finding fully appreciated, nor was the finding adequately related to the structure of the bone.

The histological identification of the 'incorporated metaphysis' has escaped recognition in the past, but Godard (1955*a, b*) observed the oblique cement line in the femur of the rat, and van der Wal (1956) described the persistence of 'filled-up substantia spongiosa' in the shaft of the metatarsus of adult cattle. The vestiges of cartilage matrix in the shaft were observed by both authors, but these structures had been previously described in human bones by Zawisch-Ossenitz (1927, 1929*a, b*).

The bottle-shaped deformity seen at the extremities of the long bones of man in diaphysial aclasis (Keith, 1920), osteopetrosis (Cohen, 1951) and familial metaphysial dysplasia (David & Palmer, 1958) may be explained as disturbances of the normal growth of the 'incorporated metaphysis'. The degree of deformity of the metaphysis in these conditions depends upon the onset of the failure of metaphysial remodelling. This latter process normally occurs immediately adjacent to the metaphysial surface of the growth cartilage (Koelliker, 1873; Leblond *et al.* 1950).

Two further points emerge when the significance of the 'incorporated metaphysis' is considered. Firstly it is important that experimental workers should not overlook the fact that there is a large portion of the definitive shaft of a growing bone where no subperiosteal deposition of bone is occurring. Secondly it is possible that this portion of a bone may be more prone to pathological change than the portion of periosteal origin or vice versa.

(4) *The 'splinting' and 'primitive diaphyses'*

There seem to be sound reasons for dividing the non-metaphysial portion of the shaft of the rat femur into two or more parts. All long bones will have a 'primitive diaphysis', but it is possible that its persistence into adult life is dependent upon the failure of metaphysial incorporation at one or both extremities. The 'splinting diaphysis' is probably only found where there is metaphysial incorporation in a bone.

These findings in the rat, if they are applicable to man, may have some pathological significance in that the otherwise inexplicable condition of progressive diaphysial dysplasia may well be due to a disturbed growth of the 'splinting diaphysis'. The

condition was described by Nehauser, Schwachman, Wittenborg & Cohen (1948) and features symmetrical spindle-shaped thickenings of the shafts of the long bones. It is of interest to note that these thickenings did not appear in the long bones of the hand and foot where one would not expect to find a 'splinting diaphysis', that when found they were always adjacent to the metaphysis of the growing end of the bone, and that there were two thickenings of the tibia which would be explicable if there had been a metaphysis incorporated at either end of the bone.

(5) *The arrangement of fibres in bone matrix in relation to bone growth*

Weidenreich (1923, 1930) reviewed the literature on the fibrous structure of bone and grouped bone into several classes, according to whether its fibres were coarse or fine and as to whether the bundles were irregular (woven) or regular (licitor bundle and lamellar). There does not appear, however, to be any account of what determines these arrangements of the fibres in the bone matrix or as to how the latter occurs. The present investigation has indicated that a bone consists of a mosaic of different types of fibrous matrix and the bone itself is undergoing constant change in structure. It would seem therefore that a dynamic approach to bone structure is essential for the full understanding of the latter, and there follows an attempt to correlate the type of bone deposited with the situation and the rate of growth of the bone.

The perichondrial bone is laid down on a smooth surface and probably is not subjected to extraneous tensions before it is calcified. Under these conditions the fibres are formed perpendicular to the surface and parallel to each other. When bone is deposited upon an irregular surface, as in the case of endochondral bone and neonatal periosteal bone, the fibres are invariably irregularly arranged. Another environmental influence on the fibre arrangement is the incorporation of extraneous fibres. These fibres pass from the bone matrix to the fibrous periosteum and no doubt cause tension differences in the bone matrix as it is being laid down about these fibres. The persistent deposition of woven bone in the 'primitive diaphysis' into the adult will be due to the presence of these extraosseous fibres.

The rate of growth of the bone, which is determined by the activity of the growth cartilage, directly influences the structure of the bone matrix. This is due presumably to the concomitant change in rate of bone deposition. When in rat bones the rate of growth is rapid, as during embryonic and early neonatal life (Bhaskar *et al.* 1954), the bone is finely fibred. However, as the rate of growth slows down the fibres collect into plaited bundles. If at the same time the surface upon which deposition is occurring is smooth, then the fibre bundles are parallel (licitor bundle bone of Weidenreich, 1930). As the growth of the bone comes to a gradual halt, the licitor bundle bone deposition is followed by lamellar bone deposition. Sissons (1953) has shown that the lower femoral growth cartilage of the rat grows at a rapid rate until 50 days, when it slows down, so that by 80 days there is little growth. As already pointed out, this latter finding coincides with the appearance of lamellar bone in the femur. Amprino (1947) suggested that there was a close relationship between the structure of bone and the rate of bodily growth. The latter factor appears to underlie the different appearances of the lamellar bone. That is, the feathered type of lamellar bone forms while the body weight would be still increasing, and possibly

to some slight extent after this has ceased, but the non-feathered type of lamellar bone does not appear in any amount until such time as the body weight would be expected to be almost constant.

SUMMARY

1. Structural changes which occur during the period between birth and 322 days are described in the shaft of the femur of the rat.
2. The bone formed during the neonatal period is found to resemble that formed in late foetal life.
3. There is a normal clubbing of the distal metaphysis which is associated with the appearance of a secondary centre of ossification, the metaphysis at this stage is unsupported by periosteal bone.
4. There is a later incorporation of the tissues of the distal metaphysis into the shaft. This 'incorporated metaphysis' contains cartilage vestiges and is separated from the rest of the shaft by a cement line.
5. A 'splinting diaphysis' is described which overlaps the 'incorporated metaphysis', and has a characteristic fibre structure.
6. The more proximal portion of the shaft which is referred to as the 'primitive diaphysis', retains its immature fibrous structure.
7. As longitudinal growth slows there is a subperiosteal and endosteal deposition of lamellar bone.
8. The proximal metaphysis does not become incorporated into the shaft.
9. These findings are discussed in relation to certain disorders of bone growth.
10. The fibrous structure of bone is correlated with the site of its deposition, the rate of bone growth, and the rate of bodily growth.

The author wishes to acknowledge the helpful criticisms of Prof. J. D. Boyd. He is also very grateful to Mr R. A. Parker for technical assistance and to Mr J. F. Crane for the photography.

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EXPLANATION OF PLATES

All the photomicrographs ($\times 1040$) are taken from $4\ \mu$ longitudinal sections of the shaft of the rat's femur, following an abbreviated Long's silver impregnation and counterstaining with neutral red.

PLATE 1

Fig. 1. 'Primitive diaphysis', 84 days. Note the irregular arrangement of the mixed coarse and fine fibre bundles.

Fig. 2. 'Primitive diaphysis', 157 days. Similar fibre arrangement to fig. 1.

Figs. 3-5. 'Primitive diaphysis', 322 days. These show the transition from the coarsely and irregularly fibred bone (fig. 3) to the lamellar subperiosteal bone (fig. 5). The transitional zone (lower part of fig. 4) is characterized by an irregular longitudinal and circumferential grouping of the fibre bundles.

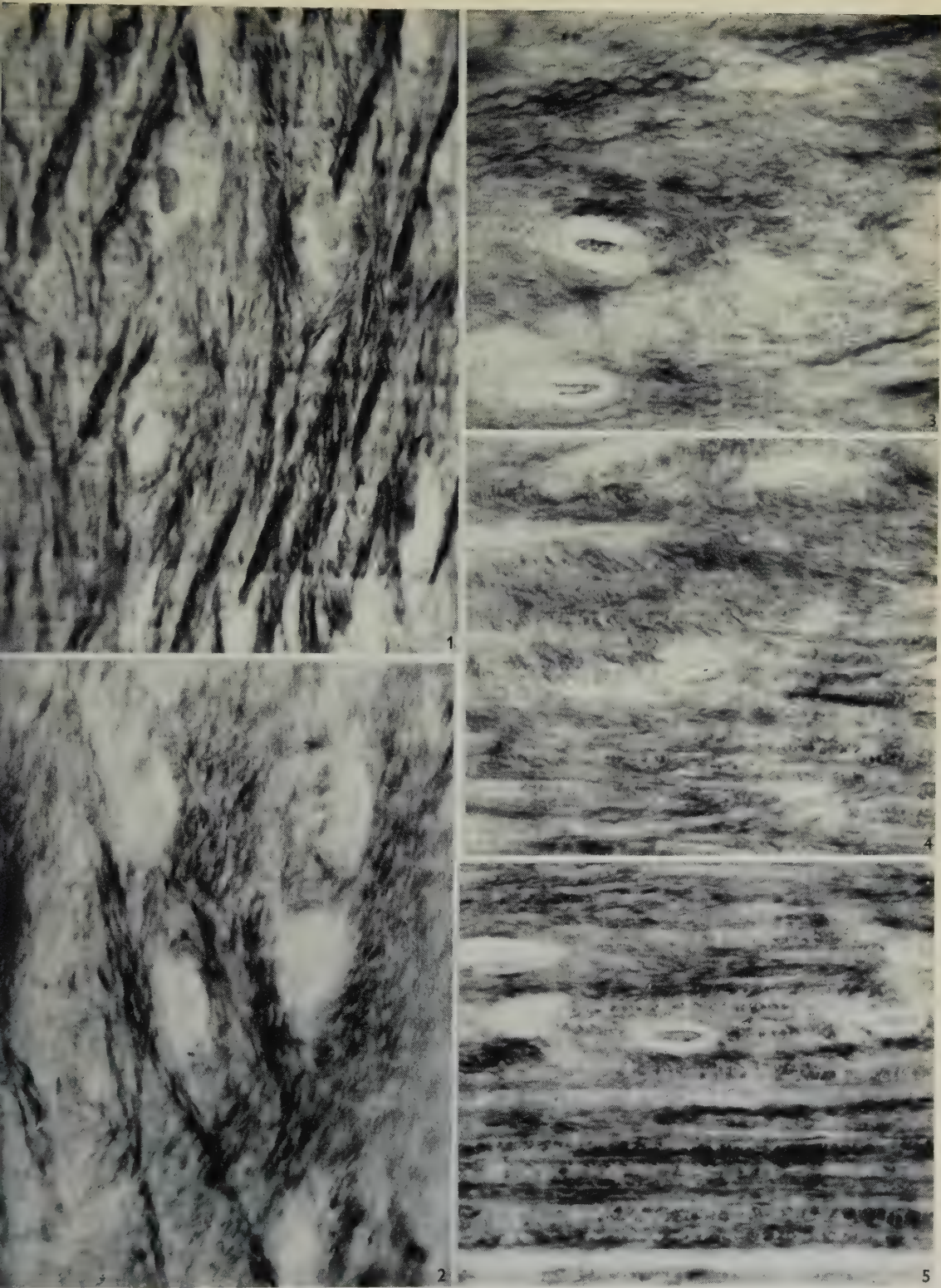
PLATE 2

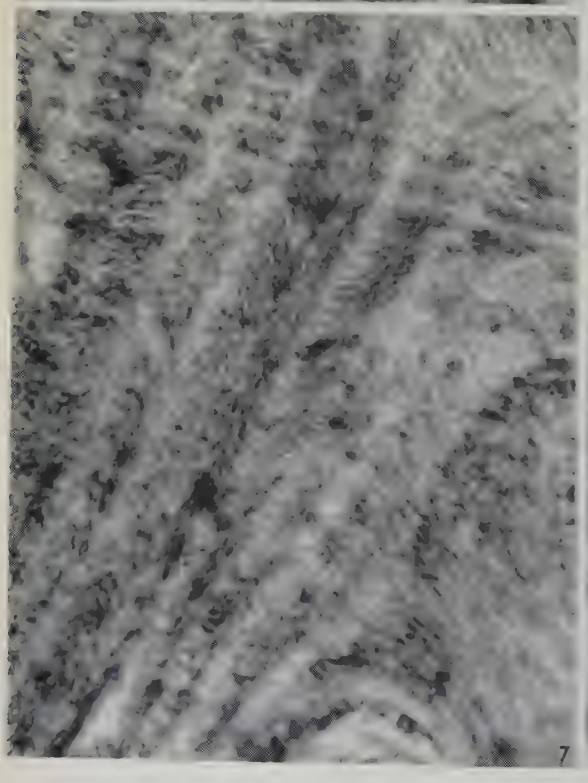
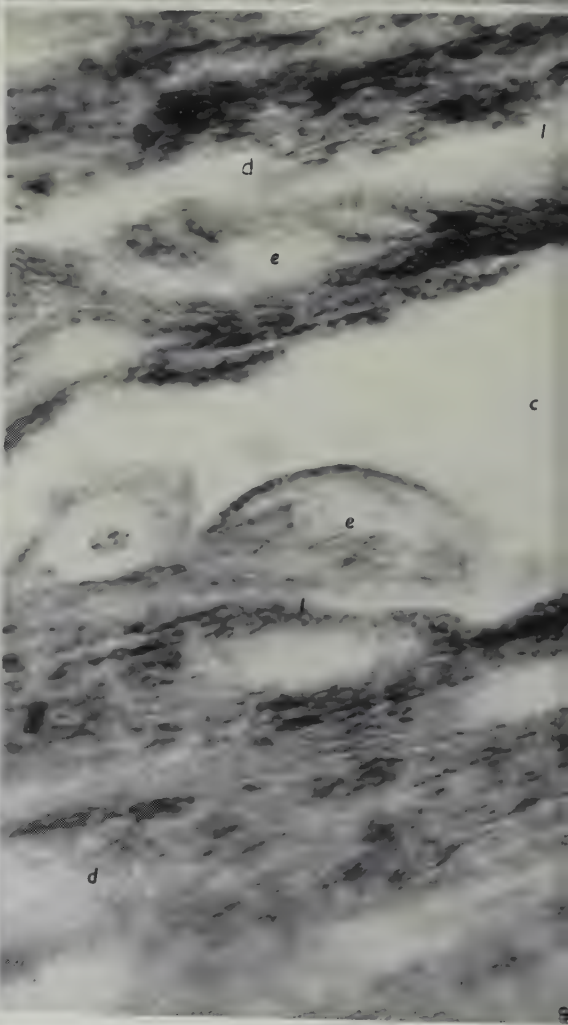
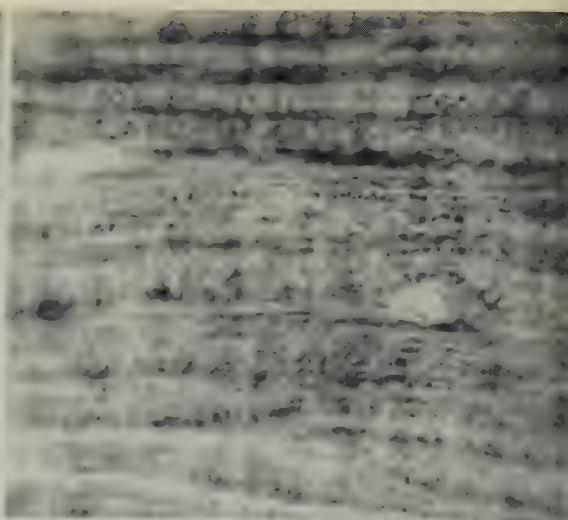
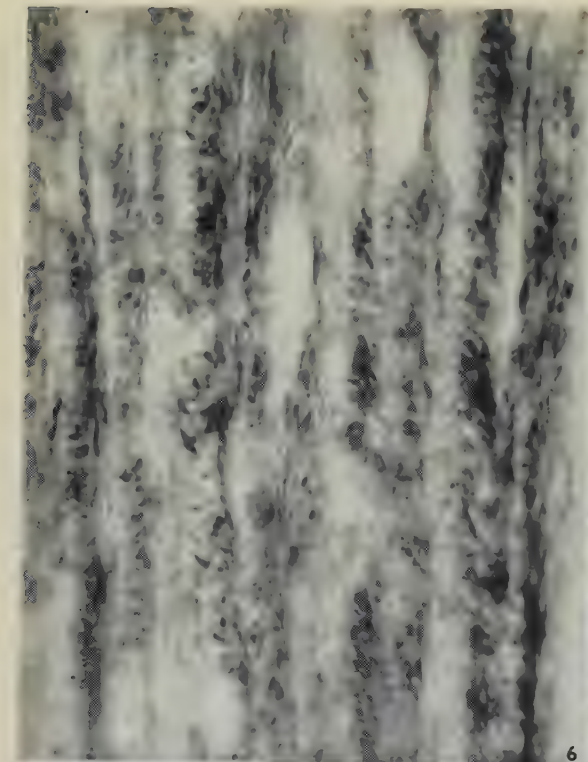
Fig. 6. 'Splinting diaphysis', 157 days. Note the feathered appearance of the lamellar bone.

Fig. 7. Metaphysis, 322 days. This lamellar bone is laid down within a vascular space, and has a feathered appearance.

Fig. 8. 'Splinting diaphysis', 322 days. The lamellar bone consists of alternating layers of longitudinal and circumferential fibre bundles.

Fig. 9. 'Incorporated metaphysis', 322 days. Note the vestige of cartilage matrix (*c*), endochondral bone (*e*), separated in places by a cement line (*l*) from the endosteal bone (*d*).





PRATT—POSTNATAL CHANGES IN THE SHAFT OF THE RAT'S FEMUR

A HISTOLOGICAL STUDY OF THE NAIL REGION IN NORMAL HUMAN SUBJECTS AND IN THOSE SHOWING SPLINTER HAEMORRHAGES OF THE NAIL

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INTRODUCTION

Prior to undertaking a histological investigation to determine the microscopic appearance, location and possible source of the so-called 'splinter haemorrhages' of the nail, a detailed study was made of the soft tissues adjacent to the nail plate (perionychium) in normal subjects, and from this investigation a number of observations concerning the normal histological features of the region were made which warrant description and comment.

In a recent clinical study of splinter haemorrhages (Platts & Greaves, 1958), it was found that though they occur most commonly in subacute bacterial endocarditis and in mitral stenosis (conditions in which they are well known to be a feature), they do in fact occur in a wide variety of conditions and are not uncommon in otherwise normal individuals. In the latter, they usually occur following minor trauma to, or jarring of the fingers, even without direct trauma to the nails: they are sometimes, but not always, accompanied by discomfort and the individual may be unaware of their presence.

Macroscopically, 'splinters' appear as tiny linear structures, usually not above 2-3 mm. long, and are arranged in the long axis of the nail. They were found to originate within the distal one-third of the nail, in relation to a pink (vascular) line, situated about 4 mm. proximal to the tip of the finger (Pl. 1, fig. 1), and they may be multiple. When first formed they are plum-coloured, but in a day or two, darken to brown or black. The splinters move distally with the growth of the nail and eventually can be scraped from its under surface: splinters so removed were found to give positive colour tests for altered blood, for example, with the benzidine, cyanol and leuco-malachite-green tests.

The nature of splinter haemorrhages has been a matter of conjecture, but it has been assumed that they are vascular phenomena, either small haemorrhages or minute emboli. Although they are often found in conditions in which vascular emboli are a feature, suggesting that they too may be embolic in nature (Platts & Greaves, 1958), the fact noted above, that they may occur in healthy subjects following minor trauma to the fingers is more suggestive of a haemorrhagic origin. It is very doubtful whether they are of the nature of petechiae however, since, as shown by Hess's test, the capillary fragility is not increased in cases of splinter haemorrhage (Platts & Greaves, 1958).

MATERIALS AND METHODS

The terminal segment of a normal finger was removed from each of ten cadavers at post-mortem, and one from each of ten cadavers showing splinter haemorrhages of the nail; the latter specimens were taken from cases which had suffered a variety of terminal illnesses, though none were available from cases of subacute bacterial endocarditis.

In the past, it has been usual to decalcify the terminal bony phalanx prior to sectioning a finger end, but it is preferable to remove the phalanx if this can be achieved without damage to the nail bed. In a recent study, Lewis (1954) removed the phalanx by drilling it out from the volar aspect of the finger by means of a special burr driven by a dental drill. The method used in the present study was to soak the finger end for several days in Heidenhain's 'susa' fixative, and then dissect out the terminal phalanx by first making a complete mid-line incision through the finger end, cutting through the nail and soft tissues down to the phalanx, and finally dissecting the two halves of the finger end completely away from the phalanx. After several more days' fixation in 'susa', the two halves of the finger end were embedded in paraffin wax and one, or occasionally both, were sectioned at $12\ \mu$ on a sledge microtome. As a routine, every tenth section was mounted and stained with haematoxylin and eosin, intervening sections being examined when indicated. With one exception, all specimens were sectioned longitudinally; the single specimen sectioned transversely contained a splinter haemorrhage and showed some interesting features.

Although splinter haemorrhages of the toe nails do occur, they were not studied histologically.

RESULTS

Before dealing with the features shown in cases of splinter haemorrhage, it is necessary to indicate the terminology used in the present work with reference to the soft tissues adjacent to the nail plate and also to deal with the relevant histological features of the region.

(a) The normal histology of the nail region

The appearance of a normal finger end in longitudinal section is shown in Pl. 1, fig. 2. The nail plate (*N*) tapers posteriorly to the nail root, which is overlapped by the proximal nail fold (*P*). The deeper layer of the epithelium of this fold is narrower than the superficial layer and differs from it in not possessing obvious papillae. Where the stratum corneum of this fold comes into contact with the nail it is called the eponychium (*E*), and this, together with the stratum granulosum, passes back along the dorsal surface of the nail to within 1 mm. of the nail root; at this point both terminate and the dorsal matrix begins (*DM* in Pl. 1, fig. 3). The distal free portion of the nail lies on a part of the stratum corneum of the finger tip, called the hyponychium (*Hy* in Pl. 1, fig. 2); together with the stratum granulosum, this extends for only a short distance under the nail; in the specimens examined, it terminated on an average 4 mm. proximal to the finger tip. Immediately distal to the hyponychium is a surface depression (*F* in Pl. 1, fig. 2) called the distal limiting furrow (Lewis, 1954).

The main body of the nail rests upon the epithelium of the nail bed, the proximal part of which is differentiated as the volar matrix (*VM* in Pl. 1, fig. 3) and this roughly corresponds in extent with the lunule of the nail. Apart from the matrix area, the nail-bed epithelium is of the same structure as the stratum germinativum (Malpighian layer) of the epidermis, but instead of dermal papillae below it there are longitudinal rows of dermal ridges; these, together with the corresponding epithelial ridges, were well seen in longitudinal sections taken from near the side margin of the finger end, because, due to the curvature of the nail and nail bed, they are here cut tangentially (Pl. 1, fig. 7). As the epithelial ridges are traced from the finger tip towards the lunule, they decrease in height and increase in number by splitting (Horstmann, 1957): this fact can be appreciated by comparing figs. 5 and 6 of Pl. 1.

The epithelium of the volar matrix differs from that of the remainder of the nail bed. In the majority of sections studied, the volar matrix had split during sectioning into two distinct zones of about equal thickness (Pl. 1, fig. 3). These two zones are structurally different; the cells of the deeper zone are polygonal, have weakly eosinophilic cytoplasm and rounded vesicular nuclei, whilst those of the superficial zone are flattened, have deeply eosinophilic cytoplasm and dark, shrunken, irregular (pyknotic) nuclei (Pl. 1, fig. 4). The cells of the upper, deeply eosinophilic zone have a matted or faintly fibrillar appearance, due to the presence of tonofibrils, and from the superficial layer, cells can be seen entering the nail plate. Intercellular bridges or 'prickles' could be distinguished in this zone, but they were shorter and less distinct than those of the general stratum germinativum of the nail bed.

Regarding the connective tissue component of the nail bed, Horstmann's (1957) observation, that the collagen fibre bundles are arranged mainly in a criss-cross lattice arrangement and have scarcely any elastic fibres mixed with them, was confirmed. In the present study elastic fibres were found only among the collagen bundles of the volar aspect and tip of the finger end. Sweat glands were not seen in the connective tissue of the nail bed but were very numerous in the volar aspect and in the tip of the finger end as far as the hyponychium region. Lamellated corpuscles (Vater-Pacini) were also numerous in the same regions.

The blood vessels of the nail bed are of particular interest. The veins are thin-walled and are connected with simple capillary loops which project towards the nail-bed epithelium (Pl. 1, fig. 10). The arteries were found to be of a special type, in that they have an inner longitudinal and an outer circular coat of smooth muscle, and have no internal elastic lamina (Pl. 1, fig. 8). Arterio-venous anastomoses, of the type known as glomi, are well known to be present in the connective tissue of the nail bed. In the specimens studied, they were found not only in the volar aspect and tip of the finger end, but throughout the connective tissue of the nail bed, even below the volar matrix, but none was seen in the proximal nail fold. Reference to fig. 9 of Plate 1 shows the manner in which a glomus is formed from an arterial branch: the branch becomes much coiled and surrounded by concentric layers of connective tissue, whilst the smooth muscle cells of its wall undergo a peculiar epithelioid change. The lumen of the structure often appears to be practically obliterated in histological sections.

In the region just below the hyponychium, vessels of a very particular kind are found. They have been described and illustrated by Horstmann (1957). In this region, the subungual dermal ridges give way to large papillae, two to three of which are seen in each longitudinal section (*L* in Pl. 1, fig. 2); they are referred to by Horstmann (1957) as the 'plump' papillae. These papillae contain long, thin-walled looped vessels of large calibre, which are spirally wound. Horstmann (1957) describes them as capillaries and likens them to heating spirals. It is difficult to say whether they should be referred to as veins or capillaries, but for convenience they will be referred to in the present work as capillaries. Examination of the finger ends from cases with splinter haemorrhages revealed that it is these vessels which rupture to give rise to the haemorrhages.

(b) The histological features in cases of splinter haemorrhage

In section, splinter haemorrhages showed as irregular amorphous masses, varying in outline and size. They were yellowish in unstained sections, and remained yellow after the sections were stained with haematoxylin and eosin, but they gave the typical bluish-green colour reaction for altered blood when treated by the cyanol method.

In all cases, the 'splinters' were located in the hyponychium, lying in the angle between the nail plate and the termination of the stratum granulosum (*S* in Pl. 2, figs. 15, 16, 17). A few splinters were found nearer to the tip of the nail and were presumably growing out with it, but in no instance was a splinter found proximal to the termination of the stratum granulosum and hyponychium. Measurements showed that the stratum granulosum and the hyponychium terminate on an average 4 mm. proximal to the finger tip. These histological observations are in keeping with the clinical observation reported above, that splinter haemorrhages first appear about 4 mm. proximal to the finger tip.

In most specimens studied, the blood vessels of the nail bed and finger tip were distended and packed with blood cells (*B* in Pl. 2, figs. 15, 16). Due to this factor, the looped, spirally wound vessels in the large papillae below the hyponychium were clearly demonstrated. In a few sections, the looped vessel was seen to terminate below the epithelium in a closely wound knot (*V* in Pl. 2, fig. 11), but far more commonly, the vessel, after a spiral wind, terminated in a simple loop (e.g. *V* in Pl. 2, fig. 13). These vascular loops, in addition to being distended with blood cells, were frequently seen to project towards the hyponychium, with very few layers of epithelial cells overlying the loop (Pl. 2, figs. 12 and 13) and, in a few instances, a vascular loop actually projected directly into the hyponychium (Pl. 2, fig. 14).

Microscopic evidence that a splinter, which appears histologically as an amorphous mass, is developed from a haemorrhage was seen in one instance, and that was in the finger end which was sectioned transversely. The distal part of the splinter in this specimen had the usual appearance of a yellowish mass (*S* in Pl. 2, fig. 18), but to one side of it a small collection of red blood cells (*R*) lay in a space in the hyponychium. In more proximal sections, a small vessel (*V* in Pl. 2, fig. 19) was seen in close proximity to the hyponychium and was presumably the source of the small haemorrhage. The proximal portion of the splinter itself was of mixed composition, part of it being composed of the usual yellowish material (*S* in Pl. 2, fig. 19).

and the remainder composed of a mass of red blood cells (*R*): in some sections the two components were intermingled (Pl. 2, fig. 20). Further proximally still, the mass was composed solely of blood cells, which could be traced into the epithelium below the hyponychium, and here a vessel was present, lying in the centre of the mass of blood cells (Pl. 2, fig. 21). Silver impregnation of adjacent sections showed that the red cell mass lay in a space in the epithelium, with no argyrophil fibres enclosing it: the small central vessel possessed the usual fibrous adventitia.

DISCUSSION

The terminology used in this work with reference to the soft tissues adjacent to the nail plate follows common usage. It may be noted, however, that Horstmann (1957) applies the term 'hyponychium' to the whole of the epithelium underlying the nail plate, whereas this region is usually referred to as the epithelial component of the nail bed, and he applied the term 'Sohlenhorn' to the tissue usually referred to as the hyponychium. Yet, he applies the term 'eponychium' in the usual way to the thin layer of cornified tissue which overlies the base of the nail. It is doubtful whether Horstmann's application of the terminology is as logical as that usually employed.

No special study was made of the nail plate in this investigation, since this has received detailed attention by Lewis (1954) and Horstmann (1957). Certain observations on the normal histology of the adjacent soft tissues were made, however, which warrant comment. It was confirmed that the nail matrix extends for a short distance on to the dorsal aspect of the nail root (Lewis, 1954; Horstmann, 1957), and that this dorsal matrix terminates where the stratum granulosum of the proximal nail fold begins: Horstmann found it to be 0.8 mm. long in a child's nail, and in the present study it was found to be 1.0 mm. long in the adult. The length of the matrix on the deep aspect of the nail root (volar matrix) measured 5 mm. on an average in the specimens studied. Horstmann (1957) states that there is not a clear demarcation between the volar matrix and the remainder of the epithelium underlying the nail plate, but this was not confirmed; on the contrary it was found that the demarcation was readily seen, due to the fact that the epithelium of the volar matrix differs from that of the remainder of the epithelium deep to the nail plate in possessing two distinct zones of cells, which usually separate during sectioning.

The structure of the blood vessels of the finger end is of interest. It was found that the arteries possess an inner longitudinal and an outer circular coat of smooth muscle and have no internal elastic lamina. Arterio-venous anastomoses (glomi) are known to be common in the finger tip and connective tissues of the nail bed, and in the present study they were found to extend proximally deep to the volar nail matrix, but were not seen in the proximal nail fold. They are generally believed to act as temperature regulators by controlling blood flow. The veins of the region are thin walled; those below the nail-bed epithelium are connected to simple capillary loops but those below the hyponychium region are connected to loops which are long, of wide bore and spirally wound. Horstmann (1967) suggests that the latter, which he likens to heating spirals, may be concerned with temperature regulation at the extremities of the fingers, and that the blood flow through them is controlled by the arterio-venous anastomoses of the nail bed.

From the present study, it is clear that so-called splinter haemorrhages of the nails are indeed haemorrhagic in origin, and that the source of the haemorrhages is from the special, spirally wound capillary loops in the large papillae which lie below the hyponychium. These vessels undoubtedly produce the pink line which is normally seen macroscopically under the nail, about 4 mm. proximal to the tip of the finger, and in relation to which splinter haemorrhages were observed to occur clinically.

In the majority of specimens examined from cases of splinter haemorrhage, there was considerable engorgement of the vessels of the finger end with blood cells, and in many instances the special capillary loops were not only distended with blood cells but were seen to be separated from the hyponychium by only a few layers of epithelial cells, and on occasions to be in direct contact with it. It is not clear whether the capillary loops are brought to this close proximity with the hyponychium by vascular congestion or whether it is an inherent feature which predisposes the individual to suffer from splinter haemorrhages, particularly if the vessels become dilated. It is evident, however, that these long, thin-walled vessels would be particularly liable to rupture if distended, even from relatively minor trauma. That they rupture into the hyponychium is not surprising, in view of their close proximity to it. Although no emboli were seen in the vessels of the specimens examined, the possibility has not been excluded that in some instances a small embolus may lodge in a vessel prior to its rupture.

After the blood has entered the hyponychium it undergoes a change, and becomes transformed to an amorphous mass, yellow in colour when viewed in section, but giving the usual colour reactions for altered blood. It is probable that the blood soon undergoes transformation after entering the hyponychium. This is suggested by the clinical observation that the haemorrhages, which are plum-coloured when first formed, become brownish or black within a day or two, and would account for the fact that in only one specimen of the series studied was an actual haemorrhage found, and even in this case part of the haemorrhage had already undergone the change to amorphous yellowish material. A clinical parallel to this example was a case of a recently formed splinter haemorrhage, observed by one of us (M. M. P.), in which the proximal part of the splinter was fluid in nature and faded on pressure, whereas the distal part was unaltered by pressure.

Regarding the nature of the material composing the splinters, it was thought that it might be related to the substance known as ceroid. This substance is described as an acid-fast brown pigment, which appears as yellow globules in sections (Lillie, 1954), and one variety of it is produced when free red blood corpuscles come into contact with fatty material; in fact, Hartroft (1951) has produced it experimentally from these sources, both *in vitro* and *in vivo*, and he has also shown (Hartroft, 1952) that it is a constituent of atheromatous plaques (haemoceroid). The stratum corneum contains fatty material (Cowdry, 1932), so that, theoretically, contact between free red blood corpuscles and this tissue might produce ceroid. Some of the tests which are used for the demonstration of ceroid (see Lillie, 1954) were therefore carried out on a few sections of splinters. It was found that they are not sudanophilic, whereas sudanophilia is a characteristic of ceroid. Nevertheless, they show some of the characteristics of ceroid, namely, they are acid-fast (to carbol fuchsin), they give

no reaction with Perl's test for iron, and with the periodic acid-Schiff (PAS) test small areas of the splinters react. Thus, the results of the tests applied, though somewhat equivocal, suggest that the material may be related to ceroid.

Although in the present study, splinter haemorrhages were seen only within the distal one-third of the nail, haematomata have been described as occurring proximal to this region, and such extravasations become incorporated into the nail substance (Alkiewicz, 1933). Recently, Loewenthal (1958) described four cases of psoriasis of the nails in which splinter haemorrhages occurred and, although they were distal to the lunule, it is apparent from his illustration that at least some of them were well proximal to the hyponychium area. He found that they became incorporated into the nail substance, which suggests that the general nail-bed epithelium as well as the matrix contributes to nail formation. On removing a splinter haemorrhage from the nail substance he found that it gave a positive benzidine test for blood, and he considered the haemorrhage to be caused by rupture of dilated capillaries of the nail bed.

It is concluded therefore that, whereas splinter haemorrhages normally occur deep to the distal one-third of the nail, after direct trauma or in disease of the nail itself they can occur between the nail and nail bed, and then they become incorporated into the nail substance. In local disease conditions, however, it is likely that the mechanism of formation is different from that described in the present work.

SUMMARY

1. Sections from normal adult finger ends as well as from those showing splinter haemorrhages of the nail have been studied, and the normal histology of the region is described.

2. Splinter haemorrhages were found to lie in the hyponychium, in the angle between the nail plate and the termination of the stratum granulosum. They are amorphous and yellowish in section, but give colour reactions for altered blood. One splinter was composed partly of yellowish material and partly of blood cells. It is suggested that the splinters may be composed of a material related to ceroid.

3. The vessels from which the haemorrhages originate are the large spirally wound capillaries which lie in the large dermal papillae deep to the hyponychium.

We should like to record our thanks to Prof. F. Davies for his helpful criticism in the preparation of the manuscript, to Mr J. H. Kugler and Miss C. J. Crockford for their technical assistance, and to Dr J. L. Edwards for the supply of post-mortem material.

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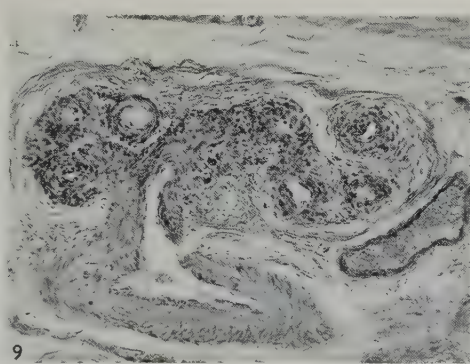
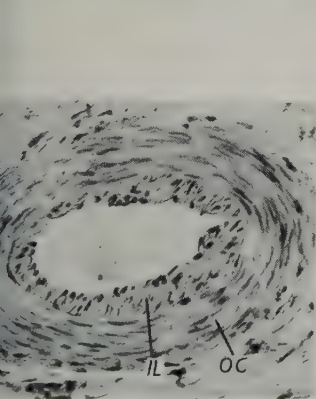
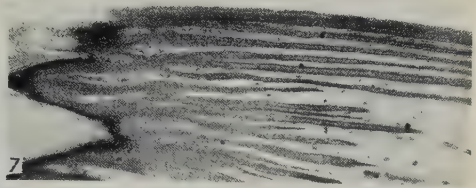
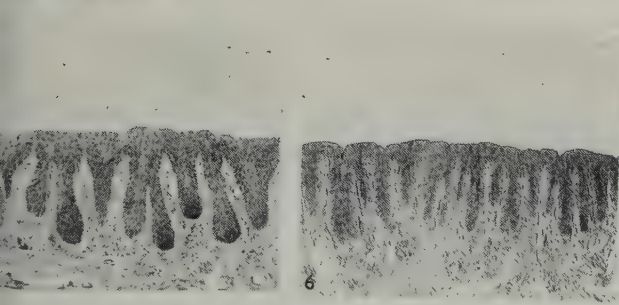
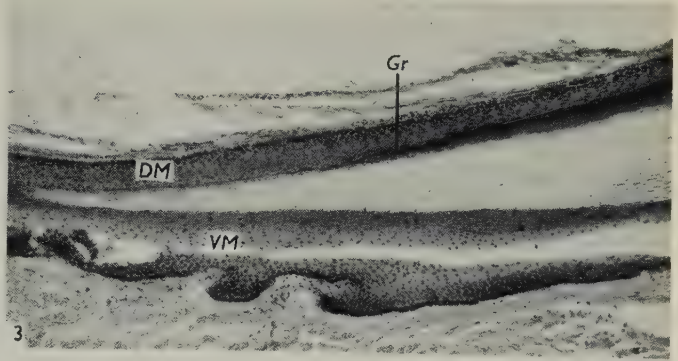
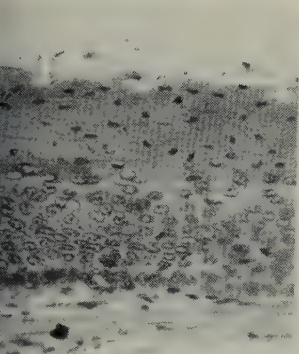
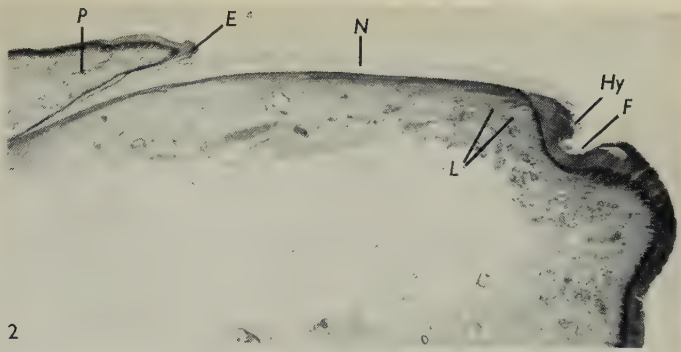
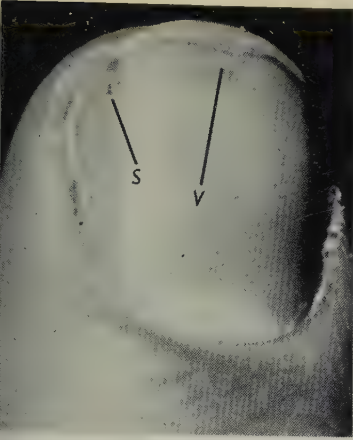
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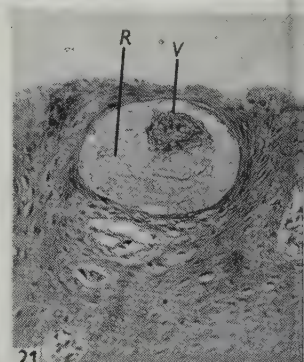
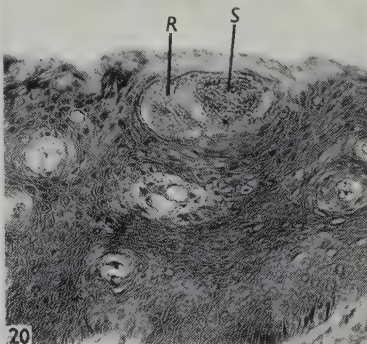
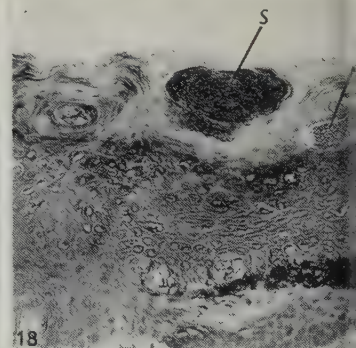
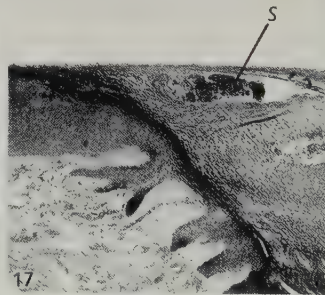
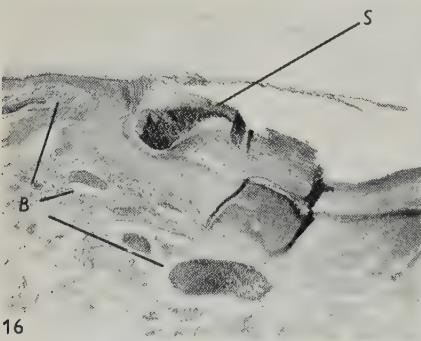
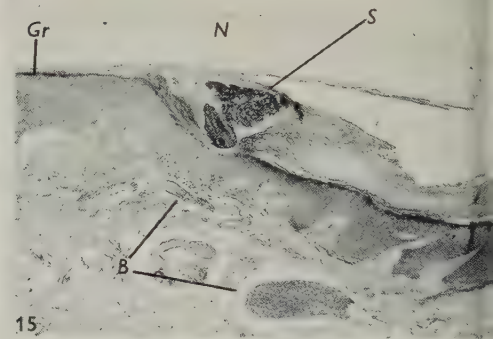
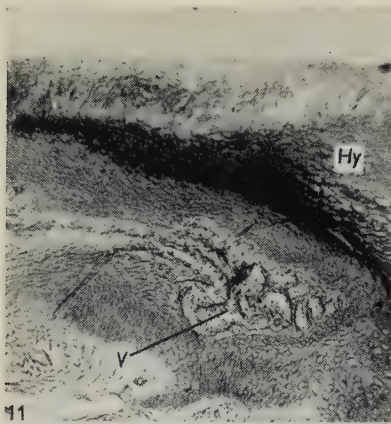
PLATE 1

- Fig. 1. Finger nail showing two splinter haemorrhages (*S*) in relation to a pink (vascular) line (*V*). $\times 3$.
- Fig. 2. L.S. adult finger end, showing nail plate (*N*), proximal nail fold (*P*), eponychium (*E*), hyponychium (*Hy*), large papillae deep to the hyponychium (*L*) and the distal limiting furrow (*F*). *H. & E.* $\times 4.5$.
- Fig. 3. L.S. through nail root. Note the termination of the stratum granulosum (*Gr*) of the proximal nail fold, the dorsal matrix (*DM*) and the volar matrix (*VM*); the latter has split during preparation into two zones. *H. & E.* $\times 54$.
- Fig. 4. Volar matrix, to show the two zones of cells; those of the upper zone are deeply eosinophilic, of matted appearance, and have pyknotic nuclei. Note cells entering the nail plate. *H. & E.* $\times 185$.
- Fig. 5. T.S. distal part of nail bed to show the epithelial ridges. *H. & E.* $\times 48$.
- Fig. 6. T.S. proximal part of nail bed. The epithelial ridges are shorter here and have increased in number by splitting. *H. & E.* $\times 48$.
- Fig. 7. L.S. from side margin of finger end, to show the subungual epithelial ridges, which have been cut tangentially. *H. & E.* $\times 20$.
- Fig. 8. T.S. artery of nail bed. Note inner longitudinal (*IL*), and outer circular coat (*OC*) of smooth muscle and absence of internal elastic lamina. *H. & E.* $\times 150$.
- Fig. 9. Arterio-venous anastomosis (glomus). *H. & E.* $\times 82$.
- Fig. 10. Simple capillary loop below epithelium of nail bed. *H. & E.* $\times 100$.

PLATE 2

- Fig. 11. Complex capillary knot (*V*) in large papilla below the hyponychium (*Hy*). *H. & E.* $\times 68$.
- Fig. 12. Capillary loop of wide bore (*V*), separated from hyponychium (*Hy*) by only a few layers of epithelial cells. *H. & E.* $\times 45$.
- Fig. 13. Large spirally wound capillary (*V*) ending in a loop, and almost in contact with the hyponychium (*Hy*). *H. & E.* $\times 68$.
- Fig. 14. Capillary loop (*V*) projecting into the hyponychium (*Hy*). *H. & E.* $\times 68$.
- Fig. 15. Splinter haemorrhage (*S*) in the hyponychium, lying in the angle between the nail plate (*N*) and the stratum granulosum (*Gr*). Vessels (*B*) are filled with blood cells. *H. & E.* $\times 23$.
- Fig. 16. Splinter haemorrhage (*S*) in hyponychium. Vessels (*B*) are distended with blood cells. *H. & E.* $\times 23$.
- Fig. 17. Splinter haemorrhage (*S*) in hyponychium. *H. & E.* $\times 23$.
- Fig. 18. T.S. finger end. Splinter haemorrhage (*S*) in hyponychium. A small collection of blood cells (*R*) lies to the right of the splinter. *H. & E.* $\times 125$.
- Fig. 19. Section proximal to that in fig. 18. The splinter haemorrhage is here composed partly of yellowish material (*S*) and partly of blood cells (*R*). The small vessel (*V*) to the right was probably the source of the blood cell collection seen in fig. 18. *H. & E.* $\times 125$.
- Fig. 20. Section proximal to that in fig. 19. The splinter haemorrhage is composed partly of yellowish material (*S*) and partly of blood cells (*R*). *H. & E.* $\times 125$.
- Fig. 21. Section proximal to that in fig. 20. The splinter haemorrhage is now composed entirely of blood cells (*R*), lying in a space in the nail-bed epithelium. A vessel (*V*) occupies the centre of the haemorrhage. *H. & E.* $\times 125$.





THE LATE INTRAUTERINE AND POSTNATAL DEVELOPMENT OF HUMAN RENAL GLOMERULI

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When examining a child's kidney, one meets the difficulty that there is no available standard, other than weight, against which to assess its maturity. The present paper reports an attempt to work out the normal standards of histological maturity of renal glomeruli during the later stages of intrauterine life and throughout childhood.

Since the beginning of the nineteenth century much work has been done on the structural development of the kidney, but most of it has been concerned with animals other than man. Herring (1898) gave a full review of the work published before 1898. During the present century Kittleson (1917) and Arataki (1925) have studied the albino rat kidney, in which the formation of new glomeruli is known to continue after birth. They found immature forms in animals up to 50 days old, which they considered to correspond with a human age of 8 years.

It has been known for many years (Peter, 1909, 1927) that the human kidney is incompletely developed at birth but most of the work on the human kidney has been carried out on single cases or on groups of cases from limited periods of childhood. Waschetko (1914), from work on six cases aged 1 day to 5 weeks, concluded that the postnatal growth of the kidney is due to the increasing size and diameter of the tubules and glomeruli and not to the formation of new nephrons. Campos (1923) used the presence or absence of the nephrogenic zone as an indication of the degree of development of the kidney in a survey of its pathological changes in congenital syphilis, and compared his findings with those in a series of forty-three normal controls. He concluded that the persistence of the nephrogenic zone beyond full term is an indication of retardation of kidney development, but he quotes Aschoff (1911) as stating that the nephrogenic zone is present in the subcapsular layer of the kidney up to 3-6 months after birth. Tsuda (1943) described a histological survey of the foetal human kidney in which he recorded the number of layers of glomeruli seen in the cortex at four different stages of intrauterine life, and he compared the diameter of the peripheral glomeruli with that of the central ones. He stated that the first glomerulus appears when the embryo is 3 in. long. At 4-5 months, he found 3-5 layers of glomeruli; at 7-9 months 9-12 layers, and at the 10th month 10-15 layers. He also stated that in all cases the central glomeruli have a greater diameter than the peripheral ones, in some cases twice as great, but never more than this. Gruenwald & Popper (1940) examined the kidneys of thirty-nine children under 22 months of age. They were concerned with juxtamedullary glomeruli only, and classified them into four stages of maturity. They considered that all are mature by the age of 2 years, and could be taken as a good indication of the general maturity of the child. Potter & Thierstein (1943) surveyed 1000 kidneys

* Working under a grant from the Medical Research Council.

from infants dying in the perinatal period, and concluded that the presence or absence of the nephrogenic zone was closely related to the weight of the kidneys and to the infant's weight, and constituted a good measure of the maturity at birth of the infant. Roosen-Runge (1949) used a control series of twenty-two normal kidneys for comparison with a series from patients with early cerebral lesions. Eight of the controls were under 2 years old and fourteen were aged 12-45 years. From these kidneys he concluded that the cuboidal epithelium has normally disappeared from the glomerular tuft by the age of 14 months.

MATERIAL

Kidneys for the present survey were obtained from necropsies carried out by the staff of the Department of Pathology, The Children's Hospitals, Sheffield, and included a large number of infants studied through the courtesy of Dr A. J. N. Warrack at the City General Hospital, Sheffield. The total number of kidneys available for sampling was 1800 and the ages ranged from 22 weeks gestation to 15½ years.

From this material, 235 cases were examined in this survey.

SELECTION OF MATERIAL

Selection was made initially by using the post-mortem record number of the case with tables of random numbers, but after 100 cases had been selected in this way it was found that the natural age incidence of mortality had weighted the series very heavily in favour of infants under 6 months. We therefore began to select cases of 6 months and over, in chronological order of death, from the post-mortem register. In this way approximately even numbers of cases were obtained above and below 6 months of age.

Only normal kidneys were included in the survey. All sections showing recognized pathological changes were discarded.

METHOD

The kidneys were all formalin-fixed, the sections were cut from paraffin blocks and were stained either with haematoxylin and eosin, or Masson's trichrome technique.

The glomeruli were classified in six stages of maturity and counts were made of the numbers of each stage present throughout the depth of the cortex. In order to do this fairly, only those sections showing the columnar arrangement of the glomeruli clearly were used, and ten columns were counted from each case. It was found that many routine sections were unsuitable for this purpose and in these cases fresh kidney blocks were cut in such a plane that the section included a complete papilla in longitudinal section with the corresponding cortex showing unbroken medullary rays running out to the subcapsular zone. One column was regarded as the area of cortex lying between two adjacent complete medullary rays and extending from the medulla to the capsule.

The criteria used in classifying the glomeruli into six stages were as follows: and are illustrated in Pl. 1.

Stage I. The 'S'-shaped proglomerulus (or pronephron, as it is better called).

Stage II. The glomerular tuft is recognizable as such, but it is mushroom-shaped

and possesses no true vascular pole. Bowman's capsule is crescentic in cross-section and is lined by cuboidal epithelium.

Stage III. The glomerular tuft has a vascular pole, but it is not divided into lobules and is covered by a continuous layer of epithelium. Bowman's capsule is circular in section and is lined by cuboidal epithelium.

Stage IV. The glomerular tuft has expanded and lobulation is present but its epithelial covering is still continuous and dips down into the clefts between the lobules. The epithelium of Bowman's capsule is being flattened.

Stage V. Considerable growth of the capillary tuft has taken place and its epithelial covering is fragmented, being represented by groups of cells in broken pallisade.

Stage VI. The capillary tuft is in adult form, the capillary spaces in it are easily seen and its epithelial covering has practically disappeared, being represented by only a few cells scattered singly over its surface.

In addition to these six categories it was necessary to provide two others: one for abnormal involuted or scarred forms, and one for 'unclassifiable glomeruli', i.e. those in which the section included only a small group of cells from the surface of the glomerular tuft and those in which the tuft had been lost from the section, the glomerulus being represented by an empty Bowman's capsule.

Each case in the survey had a separate working sheet on which the results of the ten column counts were recorded individually and then the total number of glomeruli in each of the eight categories was expressed as a percentage of the total count.

It was found that the taking of careful blocks suitable for column counting and the searching of sections to find ten different perfect columns was so time-consuming as to limit the general usefulness of the method. For this reason the method was altered to that of battlement counts made on routine sections which included the full depth of the cortex. The results of these counts were also expressed as percentages of the eight different categories present in a total count of at least 100 glomeruli.

At the time of counting, only the post-mortem number of the case was known, but after the count had been completed, the age, weight, crown-rump length, total kidney weight, cause of death and duration of illness were obtained from the hospital post-mortem records and written on the working sheet of each case. In children younger than 2 months at death the age was expressed as gestation time plus post-natal age, but in those dying 2 months or more after birth the postnatal age alone was used.

REPRODUCIBILITY TEST

In order to test the reliability of our results we carried out a reproducibility survey. This test was designed by Dr G. H. Jowett. He selected sixteen cases from the four age groups that showed the highest individual variation in the original counts and three sections were chosen from each of these cases. These sections were named α , β and γ and on each of β and γ two columns were selected and marked. On sections α and β simple battlement counts were made, and on sections β and γ the marked columns were used for column descriptions and counts. Thus four counts were made on each case, i.e. two battlement and two column counts. The survey was arranged so that on each of four consecutive days, one of the four types of count was done on each of the sixteen cases and when the whole series had been completed

(In the early cases in the series, stages III, IV and V were included in one single group and results for these stages individually are not available. This accounts for the results given only in total in the age group 44-48 weeks and also for the discrepancies within the age and crown-rump length groups between the totals of the separate stages III, IV and V counts and the III, IV, V totals shown in the tables. The figures given for the III, IV, V, total in each age group include every case in that group.)

Age group	Stage	Weeks gestation							Months postnatal							Years									
		Under																							
		28	26-29	32	36	38	40	44	48	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	1-2	2-3	3-6	6-9	9-12	12 and over
Average percentage of glomeruli grouped in maturation stages I-VI	I	11.8	8.3	4.7	1.1	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	II	10.1	9.4	4.0	5.3	2.7	0.8	0	0	0	0.9	2.4	1.5	3.7	0	0	0	0.4	0	0.1	0	0	0	0	0
	III	12.4	13.5	16.0	2.3	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	58.4	37.7	59.7	74.5	62.9	—	—	6.5	5.3	1.1	7.2	10.7	12.6	15.5	2.9	4.3	1.7	4.0	0	0	0.4	0.8	7.4	0
	V	10.6	14.2	19.4	31.6	39.1	—	12.9	11.6	44.2	92.0	85.0	54.3	47.0	67.1	87.7	83.4	50.7	53.8	71.6	31.0	17.3	0	0	0
	III+IV+V	74.8	77.9	85.6	76.7	88.2	89.4	57.0	38.3	49.0	98.3	26.0	69.1	57.2	72.7	80.3	85.2	53.9	39.5	52.6	31.8	24.6	0	0	0
VI	0.4	0	3.0	4.3	4.6	1.7	2.5	1.2	0	1.4	24.1	40.9	22.9	16.2	85.5	47.0	31.5	68.3	74.2	100.0	0	0	0	0	
No. of cases in each age group		3	19	12	21	34	3	2	2	6	5	3	3	4	8	3	5	8	4	22	7	18	8	8	3

it was repeated in the same order. A Latin square was drawn giving the order of counting so that each type of count was preceded and followed by the other three types in turn.

The results were recorded on a separate sheet for each case, and as each day's count was completed, the sheet was folded down so that it could not be read the following day. It was then put away in an envelope containing slides α , β and γ belonging to the same case.

Each of us carried out this survey independently of the other, and neither set of results was seen until both surveys had been completed.

RESULTS

The data of the cases examined is presented in Tables 1 and 2, that in Table 1 being given according to age, and that in Table 2 according to crown-rump length.

Text-fig. 1 shows the proportion of glomeruli of stages I and II related to the gestation age. It seems that the most primitive type of glomeruli constitute about 10 % of the total before 30 weeks' gestation and that these glomeruli diminish rapidly in numbers during the next 10 weeks, constituting about 1 % at 40 weeks, i.e. normal

Table 2. *Analysis of glomerular counts according to crown-rump length*

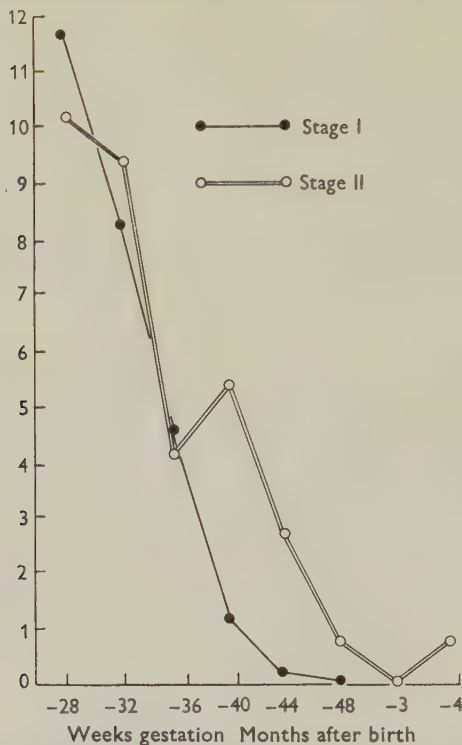
(For particulars see Table 1.)

c.r. length (cm.)	15-20	20-25	25-30	30-35	35-40	40-45	45 and over
I	13.3	6.7	7.0	0.8	0.5	0.6	0
II	4.5	6.6	8.7	1.6	0.8	2.9	0
III	12.4	22.8	6.9	4.6	0.9	16.5	0
IV	58.4	50.6	57.5	71.4	59.8	37.8	81.1
V	10.6	15.6	21.8	20.4	37.9	40.5	18.9
III+IV+V	81.4	82.8	79.3	89.1	91.3	91.7	100
VI	0	1.2	0.7	3.3	3.4	0.4	0
Total cases in each group	1	6	16	31	28	8	1

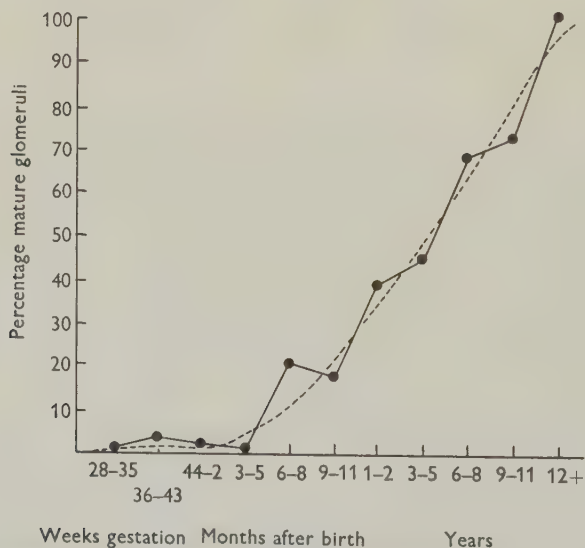
'full-term'. None were seen in normal kidneys over 46 weeks' gestation. Stage II, the slightly more organized form, which would, however, usually be called a pro-glomerulus, constitutes up to 5 % of glomeruli at 40 weeks' gestation. It seems that these normally disappear by the end of the second postnatal month. Their occasional occurrence beyond this age may represent an unrecognized abnormality.

The incidence related to age, of what appear to be fully mature (stage VI) glomeruli is presented in Text-fig. 2. This shows two fairly definite features: first, there is a nearly constant small proportion of stage VI glomeruli present, even in the most immature infants; secondly, the glomeruli are not all fully mature until the age of 12 years, i.e. approaching puberty.

Text-figs. 3 and 4 are maturity profiles at different ages, the younger age groups being represented in Text-fig. 3 and the older children in Text-fig. 4. These amplify the statements of the previous two figures, and illustrate also the general difference between the kidneys of children under one year and those over 2 years of age. In children of 44 weeks and under, the predominant glomerular type is stage IV. By 6 months after birth the predominance has shifted to stage V. This predominance



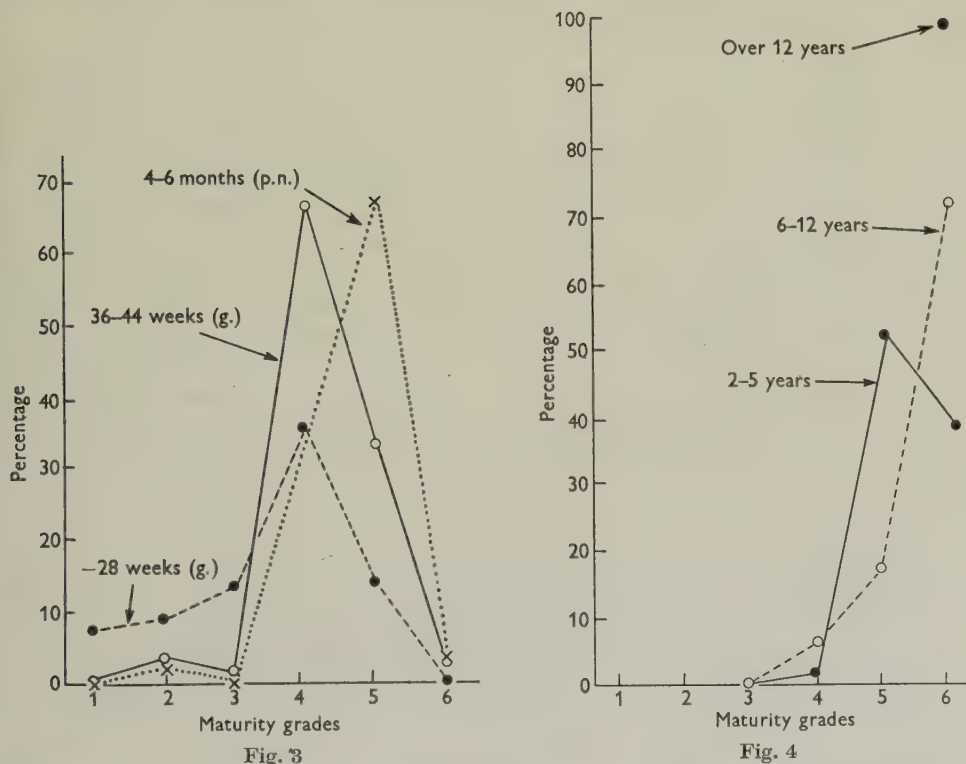
Text-fig. 1. Percentage of stages I and II present according to age. Gestation age is the period of gestation plus the postnatal age and is used in all infants of a postnatal age of less than 2 months.



Text-fig. 2. Percentage of stage VI glomeruli present according to age. There is a very small percentage of adult forms present in the most immature kidneys, but all glomeruli have reached adult form by 12 years of age.

of stage V appears to carry on throughout infancy to the age of 5 years, after which fully mature glomeruli predominate. Glomeruli of stages I and III are absent by 2 years, but glomeruli in which the epithelial covering of the tuft is present in the form of a fragmented pallisade (stage V) constitute 7% of the total in the age group 9–12 years.

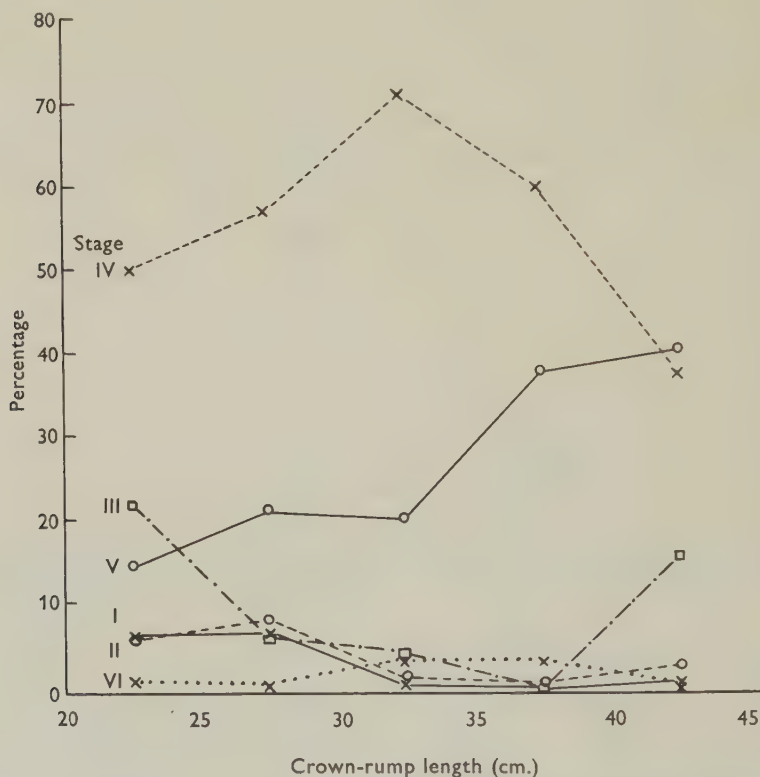
The relationship between the crown-rump length of the infant and the maturity of the glomeruli is represented in Text-fig. 5, and in Table 2. The crown-rump measurement is used as it probably gives the best single general indication of maturity in an infant. In this series, glomeruli of stage I are no longer found at a crown-rump length of about 35 cm. and the maturation of stage II seems to follow the stage I pattern very closely. Concurrently the stage III glomeruli mature rapidly, giving a steeper disappearance curve than stages I and II, until they are absent at crown-rump length 35–40 cm. Their subsequent reappearance in the



Text-fig. 3. The percentages of the six maturity grades of glomeruli present in three age groups below six postnatal (p.n.) months. At 28 weeks from conception there is a high proportion of the most primitive glomeruli (g.) though the majority are at stage IV. Between 36 and 44 weeks from conception a much greater majority are at stage IV and the percentage of primitive forms has fallen markedly. At a postnatal age of 4–6 months the great majority of glomeruli have matured to stage V and the primitive forms have virtually disappeared.

Text-fig. 4. The percentages of the six maturity grades of glomeruli present in three age groups above 2 years. Stages I, II and III have disappeared. At 2–5 years the majority of glomeruli are still at stage V though adult forms constitute a large minority. From 6 to 12 years the adult glomeruli form the great majority. From 12 years onwards, only adult forms are present.

longest infants is an unexplained finding, but it may represent a group of abnormal involuting forms. Glomeruli of stage IV predominate throughout the whole period, but seem to diminish fairly rapidly from the 30 to 35 cm. group, being replaced by glomeruli of stage V. Glomeruli of the most mature form (stage VI) are present at all crown-rump lengths in this series and all are located in the juxta medullary area, often very close to the deepest layer of the arcuate vessels.



Text-fig. 5. The percentages of the six maturity grades of glomeruli present according to crown-rump length. In general the three most primitive stages disappear at a crown-rump length of 35–40 cm. While they are present, the majority of glomeruli are at stage IV, but from 35 cm. onwards the percentage of this stage diminishes rapidly and no longer constitutes the majority by the time the crown-rump length has reached 40–45 cm.

DISCUSSION

The aim of this study was to achieve a picture of the normal histological maturity of renal glomeruli at different ages in infancy and childhood in order to be able to assess variation and diagnose particular renal immaturities. But the individual variation between cases, and, more than that, the intrinsic difficulty in attempting to put a subjective impression of histological form on to a statistical basis has, to some extent, defeated us.

The reproducibility survey showed that one of us (M. S. M.) had subconsciously altered the standard of assessment during the survey, and that there was error in

the grading of glomeruli between adjacent categories. This basic error in assessment of individual glomeruli was, fortunately, largely offset by the number of kidneys examined and by the random selection of cases, so that the general pattern described and conclusions drawn from it are likely to be as near the truth as the personal factor inherent in our method will allow.

The following pattern of development appears in the cases we have examined. Very primitive incompletely formed glomeruli (stage I) are not to be expected after the age of 44 weeks from conception, nor in infants with crown-rump length greater than 25–30 cm. In kidneys in which stage I are present, the majority of glomeruli will be at stage IV, but after stage I has disappeared the majority will be at stage V. These remain in the majority during the first 5 years of life, but from 6 years onwards stage VI predominates, though it is not until after the age of 12 that all glomeruli may be expected to be of adult form.

The number of fully mature glomeruli, sometimes found in the juxta-medullary region of kidneys still showing stage I, do not begin to increase rapidly until 3–5 years of age. The large foetal juxta-medullary glomeruli have been described a number of times and are the subject of a separate communication.

The present survey has been carried out on a somewhat different basis from that of Potter & Thierstein (1943). They were seeking a histological tool for the assessment of general maturity, whereas we have attempted to establish normal histological standards of renal maturity throughout childhood. Nevertheless, the general conclusion we have reached from our observations on the newborn infants in our series agree with theirs, though we have found a scatter in the time of disappearance of proglomeruli sufficient to make us doubtful of its value as a criterion of maturity in an individual case.

In the older children in our series we have found that full histological maturity is reached at a later age than has been generally believed.

It seems therefore that the histological maturation of the kidney may be roughly divided into three phases:

(1) The nephrogenic phase in which new glomeruli are being formed. This phase may continue until 44 weeks after conception, but is usually over by the time the infant has reached the 36th week of gestation or has attained a body length of 30–35 cm.

(2) A phase in which the full complement of glomeruli is present, but almost all are immature. This phase lasts until 3–5 years of age.

(3) A phase of final maturation which appears to extend from 3 to 12 years. During this phase there is a steady increase in the proportion of fully mature glomeruli of adult form present in the kidney.

SUMMARY

A survey has been made of the histological maturity of the renal glomeruli of 235 children whose ages ranged from 26 weeks of gestation to 13½ years.

The most primitive glomeruli are not found after the 44th week from conception and are found only occasionally after the 36th week.

Primitive forms may persist until 4–6 months of age.

The majority of glomeruli are of mature type from 6 years onwards.

It is not until after 12 years of age that all glomeruli are of adult form in all kidneys.

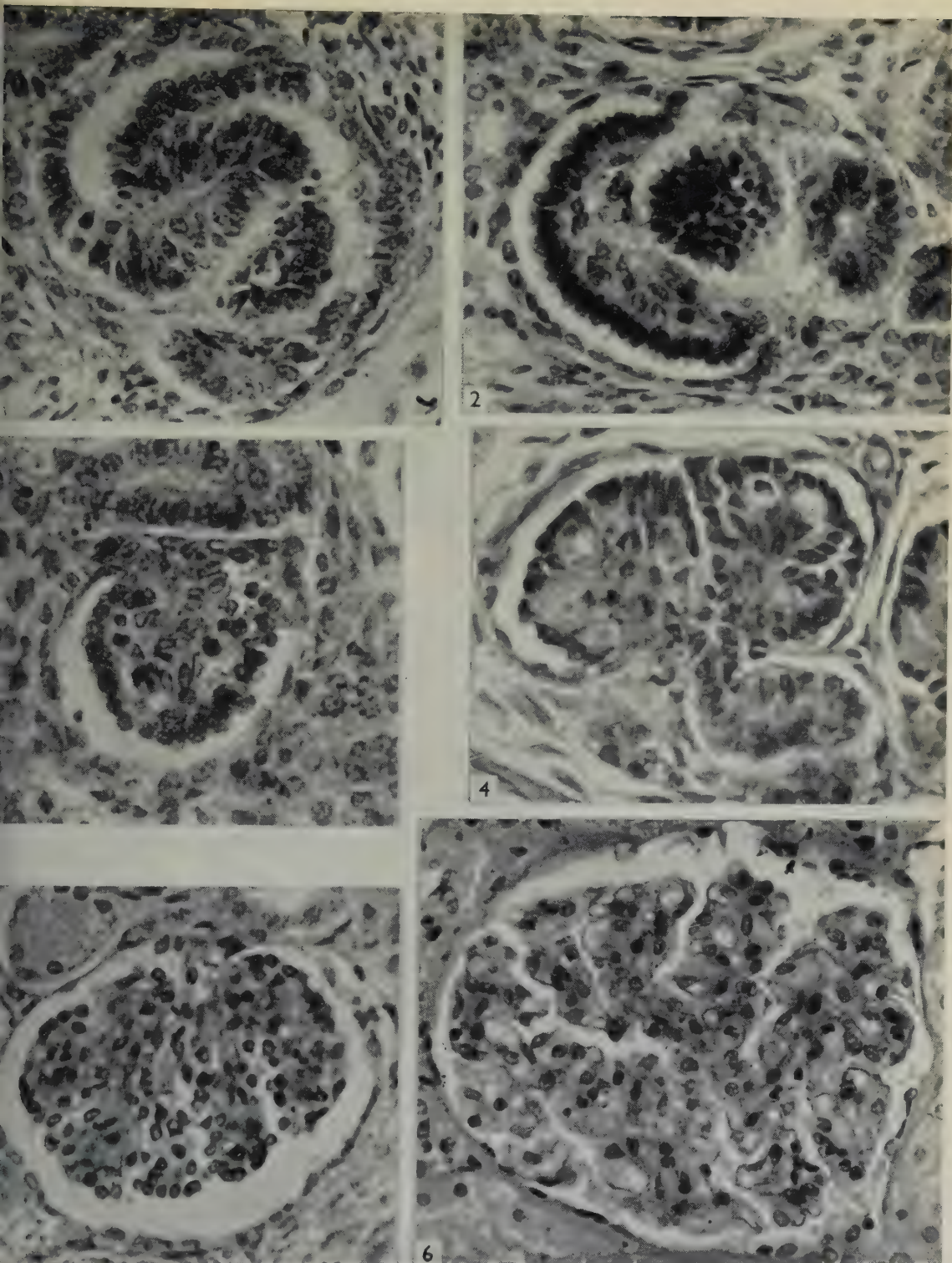
A very wide individual variation in the rate of glomerular maturation was found.

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EXPLANATION OF PLATE

Fig. 1. Stage I glomerulus. Fig. 2. Stage II glomerulus. Fig. 3. Stage III glomerulus. Fig. 4. Stage IV glomerulus. Fig. 5. Stage V glomerulus. Fig. 6. Stage VI glomerulus. All glomeruli are at the same magnification (1/500).



CHANGES IN THE DEOXYRIBONUCLEIC ACID CONTENT OF GANGLION CELLS DURING CHROMATOLYSIS

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In previous papers attention has been drawn to changes which occur in the nucleic acid content of peripheral nerve during degeneration and after stimulation (Logan, Mannell & Rossiter, 1952; Causey & Stratmann, 1956*a*), and also in ganglia during the process of chromatolysis and following stimulation (Gersh & Bodian, 1943; Causey & Stratmann, 1956*b*).

Biochemical methods of analysis as used in our previous studies (Causey & Stratmann, 1956*a, b*) can provide accurate information on the overall changes in nucleic acids throughout the tissue under examination, but give only indirect and limited information about the localization of these changes in the individual components of the tissue. For this reason microphotometric methods of analysis were employed in an attempt to localize the observed changes and to shed some further light on their significance.

In this paper results are reported of a detailed examination of the normal neurone of the superior cervical ganglion of the rabbit and these are compared with observed changes in the deoxyribonucleic acid (DNA) content of neurones undergoing chromatolysis.

MATERIAL AND METHOD

The Feulgen nuclear reaction has for many years been considered specific for DNA (Davidson, 1953) and in recent years it has been shown that the intensity of the stain may be related directly with the amount of DNA present in the cell nucleus (Di Stefano, 1948; Swift & Rasch, 1956).

The apparatus used for determinations of the intensity of staining consists essentially of a microscope, a light source and a photometer. In this study the apparatus used was a Hilger High Power Microphotometer. For routine measurements a magnification of 200 diameters was used. In the Hilger instrument the photometer consists of a photomultiplier tube, the output of which may be read directly on a milliammeter or recorded on a Honeywell-Brown recording galvanometer.

The magnified image of the section is projected onto the photomultiplier grid, the area to be measured being delimited by a diaphragm aperture of a size such that approximately two-thirds of the feature to be measured was covered. In the case of neurone nuclei the aperture diameter was 0.77 mm.

In order to measure the absorption of a nucleus, a clear area adjacent to the nucleus to be measured was chosen and a background reading taken. The feature to be measured was then moved into the area covered by the aperture and a reading again taken. The transmission (T) of the feature is then given by dividing the background reading I_o into the reading obtained from the nucleus (I_s).

Thus $T = I_s/I_o$.

E , the extinction of the feature, is defined as $\log_{10} 1/T$ or $\log_{10} I_o/I_s$.

For a large number of absorbing substances there is a straight-line relationship between E and the concentration of the absorbing substance (Beers Law). The Feulgen stain has been shown to obey this law within the limits of concentration found in nuclei (Swift & Rasch, 1956).

In measuring the absolute amount of absorbing material in spherical bodies such as nuclei, a number of important geometrical and other problems arise. However, in this study it was desired to make comparative measurements only and no attempt was made to determine absolute amounts of absorbing material present nor was any attempt made to relate the amount of absorbing material with the absolute amount of DNA present in the nucleus. The assumption which has been made is simply that the measured absorption was proportional to the amount of DNA present in the nucleus.

Diaphragm apertures were chosen of such a size that the absorption of only a central area or 'plug' (no more than two-thirds volume of the nucleus) was measured. In this way the effects of peripheral background light and marginal diffraction are reduced to a minimum (Swift & Rasch, 1956). Errors due to non-uniformity of the 'plug' measured will tend to cancel out when large numbers of nuclei are measured. In any event this and other accumulated errors, for example differences in section thickness, find expression in the standard error of mean given with each result.

All results are calculated in terms of M , the amount of absorbing substance, defined as $E \times A$, where E is the extinction, and A the area (in μ^2) of the slide actually measured. For convenience the resultant figure is multiplied by 100.

Staining procedure

After fixation and embedding, 8μ transverse sections of all material were prepared.

Sections were stained for DNA by a method substantially similar to the modified Feulgen technique described by Rafalko (1946).

In order to obtain maximum reproducibility of the technique, all stages of the staining procedure were carried out in a Histokinette tissue processor.

The several stages are detailed below:

Distilled water	2 min.
N-HCl, room temperature	2 min.
N-HCl at 60° C.	25 min.
N-HCl, room temperature	2 min.
Distilled water rinse	2 min.
Sulphurous acid	2 min.
Leuco Basic Fuchsin	2 hr.
Sulphurous acid	2 min.
Sulphurous acid	2 min.
Tap water	15 min.
Tap water	15 min.

Distilled water rinse, followed by usual mounting procedure.

No counterstaining was carried out.

The superior cervical ganglia of rabbits of about 2.0 kg. body weight were used. All survival operations were carried out under Nembutal (pentobarbitone sodium B.P.) and ether anaesthesia. Chromatolysis of the nerve cells was initiated by section of all the cephalic branches of the ganglion close to the body of the ganglion (Causey & Stratmann, 1956*b*). The animals were killed 7 to 28 days later and the specimens were dissected out immediately after death.

The ganglia were dissected out carefully, freed of fat and fibrous connective tissue as far as possible and then fixed for 5–6 hours in Zenker-Formol fixative. Subsequently, the material was washed overnight in running water, and embedded in paraffin by the usual procedure.

RESULTS

In view of the large number of variables inherent in the photometric technique, including the well-recognized difficulties encountered in reproducing exactly all stages of a complicated staining technique, considerable care was taken to establish reliable figures for the nuclei of normal neurones. In order to do this estimations were carried out to determine what variations could be detected in DNA content of the neurone nuclei taken from different levels of the superior cervical ganglion, from opposite sides of the same animal and, of course, variations between different animals.

The mean diameter of the neurone nucleus of the superior cervical ganglion of the rabbit was found to be 11.1μ (standard deviation ± 1.4). There is, of course, a difference in cell size during chromatolysis (van Harreveld, 1957), but if there is a change in nuclear size also it is not detectable at the magnification used in our experiments.

The photometer aperture diameter used for all measurements carried out on sections of the superior cervical ganglion was 0.77 mm. which, at the magnification used in these experiments (200 diameters), covered an area on the slide of $11.6 \mu^2$. The section thickness (8μ) was kept constant throughout the work.

The results of measurements of the amount of absorbing substance (M) in the neurone nuclei of the normal superior cervical ganglion is shown in detail in Table 1. In the same table results are shown for values of M at different levels of the same ganglion, and from opposite sides of the same rabbit.

For normal neurones a mean value of 85.8 with a standard error of 0.95 (503 nuclei) was obtained. It will be seen from examination of the table that no significant differences could be observed at different levels of the same ganglion, between ganglia from opposite sides of the same animal and between ganglia obtained from different rabbits. As well as expressing normal variation, the figure for the standard error can be considered to contain variations due to small differences in section thickness and random variables in the measuring technique.

For comparison purposes, therefore, the mean value of 85.8 was taken as representing the normal level of absorbing substance present in the neurone nucleus of the superior cervical ganglion of the rabbit and was considered to be proportional to the amount of DNA present.

Fig. 1, Plate 1, shows a photograph of a typical 8μ section of superior cervical ganglion taken with the interference microscope to show the type of nuclear absorption obtained by the method.

Table 1. *Normal superior cervical ganglion of the rabbit.*

(Results are expressed in terms of M , the amount of absorbing substance present in the area of the nucleus measured. Thus $M = E \times A \times 100$, where E = extinction, A = the area measured = $11.6 \mu^2$. Mean values for M are given together with the standard error of the mean. Section thickness throughout the determinations was 8μ . Number of nuclei measured is given in parentheses after each result.)

Normal neurones: $M = 85.8 \pm 0.95$ (503) (5 rabbits)

Different levels of the same ganglion. Neurones: (1) proximal level; (2) distal level; (3) middle level.

Mean S.E.M.

- (1) $M = 79.1 \pm 2.69$ (53)
 (2) $M = 80.4 \pm 2.23$ (51)
 (3) $M = 78.8 \pm 2.12$ (51)

Ganglia obtained from four different rabbits. Neurones:

Mean S.E.M.

- (1) $M = 84.7 \pm 1.65$ (103)
 (2) $M = 86.3 \pm 1.63$ (97)
 (3) $M = 85.2 \pm 2.48$ (53)
 (4) $M = 86.1 \pm 2.46$ (51)

Ganglia obtained from opposite sides of the same rabbit. Neurones:

Mean S.E.M.

- Right-hand side $M = 81.7 \pm 2.17$ (50)
 Left-hand side $M = 85.8 \pm 2.58$ (56)

In Table 2 results are shown of the determination of the amount of absorbing substance in the nuclei of neurones in which the process of chromatolysis has been initiated. It will be seen from the table that there has been a significant decrease in the level of absorbing substance (M) even at 7 days and that this decrease continues up to 14 days after section. At 21 and 28 days there is no significant further decrease and the level of M has been reduced to approximately 50 compared with 85 found in normal neurones.

Table 2. *Superior cervical ganglion of the rabbit during chromatolysis produced by section of the cephalic branches of the ganglion close to the body of the ganglion*

(Results are expressed in terms of M , the amount of absorbing substance present in the area of the nucleus measured. Thus $M = E \times A \times 100$, where E is the extinction and A = the area measured = $11.6 \mu^2$. Mean values for M are given together with the standard error of the mean. Number of nuclei measured is given in parentheses after each result. Section thickness = 8μ .)

Time after section	Mean	S.E.M.	Number of nuclei measured	Number of animals
0 hr. (normal)	85.8	0.95	503	5
7 days	64.2	4.5	100	2
14 days	49.8	2.2	86	2
21 days	54.8	3.1	100	2
28 days	50.1	2.9	102	2

The mean diameter of neurone nuclei undergoing chromatolysis was found not to be significantly different from normals at the magnification used. Therefore the observed change cannot be due to change in volume of the nucleus.

Fig. 2, Plate 1, shows the appearance of a section of the superior cervical ganglion of the rabbit, 14 days after post-ganglionic section to initiate the process of chromatolysis.

DISCUSSION

In this paper changes in the DNA content of neurone nuclei during the process of chromatolysis have been reported. Microphotometric methods of analysis have been used whereby the DNA concentration in individual nuclei can be examined. The accuracy of such methods of measurement on Feulgen-stained nuclei has been discussed at length by Swift & Rasch (1956). Determinations of absolute quantities of DNA in nuclei by such methods are exceedingly complex and subject to many sources of error. It is desired to stress, however, that in this study no attempt has been made to determine absolute amounts of nucleic acid, but rather the method has been employed to follow changes in the DNA content under various conditions. With both normal and experimental material the cytological technique has been kept as similar and constant as possible, and in this way both method and calculation have been considerably simplified.

During degeneration of peripheral nerve it has been shown that there is an overall increase in nucleic acid content of the portion of the nerve peripheral to the cut or crush (Logan *et al.* 1952; Causey & Stratmann, 1956*a*). This increase can be largely attributed to the proliferation of Schwann cells which occurs during degeneration (Abercrombie & Johnson, 1946), but there is evidence that changes also occur in the cytoplasm of the neuronal elements as well (Causey & Stratmann, 1956*a*). A similar overall increase in nucleic acid content in the whole superior cervical ganglion following section of the post-ganglionic trunks has also been observed (Causey & Stratmann, 1956*b*). Section of the post-ganglionic trunks initiates the chromatolytic cycle in the neurones but also leads to a rapid proliferation of Schwann cells within the ganglion (Barton, 1957; Barton & Causey, 1958*a, b*). The increase in both DNA and ribonucleic acid in the whole ganglion following section may be considered, therefore, as being a reflexion of this proliferation of Schwann cells. The increase in nucleic acid content, however, is in marked contrast to the fall in DNA content of the neurone nuclei themselves reported in this paper.

Rapidly metabolizing cells in general have a higher DNA content than less active ones (Davidson, 1953), and the decrease in DNA content of the neurone nuclei could possibly be explained if one visualizes a decrease in metabolic activity during chromatolysis. This would appear most unlikely, however, for the activities of the cell are directed towards regeneration of the severed fibres. The overall increase in DNA in the ganglion, bearing in mind the decrease observed in the neurone nuclei themselves, is likely, therefore, to be greater than indicated by biochemical analyses of the whole ganglion.

The actual decrease in DNA in the nucleus of the neurones may be explained perhaps by a decrease in polymerization of a fraction of the DNA in the nucleus of the chromatolysing cell. This may have the effect of making a fraction more soluble and therefore unavailable for the staining reaction. There is also the possibility that a fraction of the DNA present in the nucleus is transferred to the cytoplasm or may even be converted into ribonucleic acid (RNA), although there is no direct evidence for this. Proliferating cells show an increase in DNA content. Neurones do not proliferate and the observed fall in DNA content during chromatolysis may be another expression of this difference between neurones and other cells.

The regeneration of a nerve fibre requires considerable protein production. Hydén (1943) and Brattgård (1952) have drawn attention to the relationship between protein formation and RNA content, and it can be said that elevated quantities of RNA are required when active protein synthesis is in progress. Previous biochemical studies indicate that there is a very great increase in RNA during chromatolysis and that this increase continues longer than the DNA increase. It could be, therefore, that the greatly increased RNA requirements of the cell lead in some way to the observed fall in DNA in the nucleus. In any case the results reported would indicate that the DNA of the neurone nucleus, or at least a fraction of it, is more labile than has hitherto been supposed.

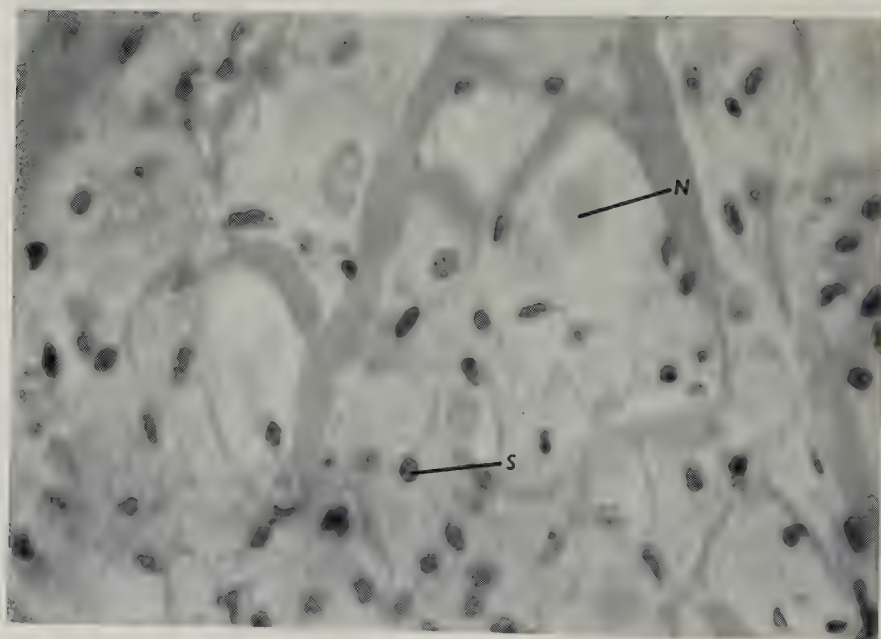
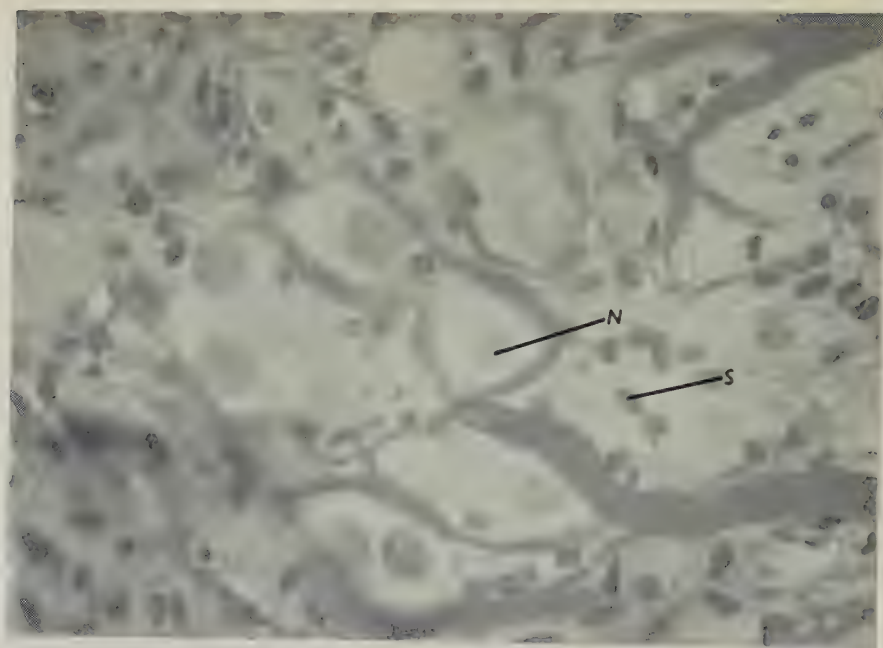
SUMMARY

1. A microphotometric method is described for comparative measurements of the deoxyribonucleic acid (DNA) content of neurone nuclei obtained from the superior cervical ganglion of the rabbit.
2. In normal nuclei the amount of DNA per nucleus was found to be constant within narrow limits.
3. During chromatolysis a substantial fall in DNA content of the nuclei is reported.
4. These results are discussed in relation to previous work in which biochemical analyses of DNA of whole ganglia were carried out.

We wish to thank Miss Joyce Armstrong for technical assistance, Mr S. A. Edwards for assistance and advice with photography, and the British Empire Cancer Campaign for financial support.

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CAUSEY AND STRATMANN—CHANGES IN DEOXYRIBONUCLEIC ACID CONTENT OF GANGLION CELLS

(Facing p. 347)

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EXPLANATION OF PLATE

- Fig. 1 (top). Feulgen stained 8μ section of normal superior cervical ganglion of the rabbit. *N* = neurone nuclei. *S* = satellite cell nuclei. Magnification = 700 diameters.
- Fig. 2 (bottom). Feulgen stained 8μ section of superior cervical ganglion of the rabbit. 14 days after section of the post-ganglionic trunks. *N* = neurone nuclei. *S* = satellite cell nuclei. Magnification = 700 diameters.

THE FORMATION OF A MUCOPROTEIN-SULPHATED MUCOPOLYSACCHARIDE COMPLEX IN THE LYMPHOID TISSUE OF THE PREGNANT GUINEA-PIG

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It has long been known that the lymphocytes of the normal guinea-pig may contain an inclusion body of a type not seen in other species. These cells, called Kurloff cells after their discoverer, increase greatly in number in the pregnant animal and were shown by Ledingham (1940) to be produced in both males and females by administration of oestrogens. Pearse (1949) studied the histochemical reactions of the Kurloff cells and concluded that the inclusion bodies were composed of mucoprotein, probably secreted by lymphocytes. The older literature on these cells has been reviewed by Babudieri (1938). The following study was performed to investigate the histogenesis and histochemistry of the Kurloff cells with regard to their possible function in the pregnant animal.

TECHNICAL METHODS

Tissues for study were obtained from male and female guinea-pigs injected with oestrogen. A dose of 0.5 mg. of oestradiol benzoate in oil per 500 g. body weight was given by intramuscular injection at 6-day intervals, the animals being killed on the 6th, 8th, 10th, 12th, 17th and 20th days after the first injection. A small group of animals was given a single injection of oestrogen and killed at intervals of from 22 to 35 days afterwards. The spleen, thymus, bone marrow, lungs and lymph nodes were removed for histological study and imprint preparations made from the cut surface of the spleen.

Tissues were fixed at room temperature in 4% formaldehyde saline, in Zenker's fluid, Susa's fixative, Carnoy's fluid, 95% methyl-alcohol formalin, 10% formalin with 4% lead subacetate, 4% formaldehyde calcium, 80% methyl alcohol and 70% ethyl alcohol. In addition, tissues were fixed in 4% formaldehyde saline at 4° C. for 4-8 hr. only, in absolute methyl-alcohol for 18 hr. at 4° C. and in 95% methyl-alcohol formalin for 3 hr. at 4° C. Imprint preparations were fixed wet in Susa's fixative for 15 min., in Carnoy's fluid for 15 min., and in absolute methyl-alcohol for periods from 15 min. to 2 days. Tissues were dehydrated in alcohol, commencing at 70% strength, cleared in chloroform and embedded.

Paraffin sections were cut at 5 and 10 μ and stained by the following methods: haematoxylin and eosin, periodic acid Schiff before and after diastase, hyaluronidase or trypsin treatment, Alcian blue at pH 0.2 before and after hyaluronidase, 1% toluidine blue before and after hyaluronidase and ribonuclease. Imprint preparations were stained by the same methods as paraffin sections. In addition, the Millon reaction, the Sakaguchi reaction for arginine, the dimethylaminobenzaldehyde-nitrite reaction for tryptophane (Adams, 1957), the performic acid Alcian

blue method for cystine (Adams & Sloper, 1956) and the ferric-ferricyanide method for reducing groups were carried out on imprints and paraffin sections.

Formol calcium fixed frozen sections were stained with the Sudan black and Baker's acid haematin methods, and fixed and unfixed imprints stained with Sudan black. Hyaluronidase extractions were performed using a testicular hyaluronidase (Hyalase, Bengel Ltd.) at a concentration of 1 mg./ml. in buffered saline at pH 7 for 18 hr. at 37° C. together with saline controls. An estimation of the methylene-blue extinction point of the Kurloff cell inclusion body was carried out by the method of Dempsey & Singer (1946), using paraffin sections and imprint preparations.

ISOTOPE STUDIES

Four male guinea-pigs treated with oestrogen and two untreated males were injected intraperitoneally with ^{35}S (A.E.R.E. Harwell) given as sodium sulphate in isotonic saline without carrier. Injections of 1 $\mu\text{c.}$ /g. body weight were given on the 8th, 10th and 12th days of oestrogen treatment, and 2 $\mu\text{c.}$ /g. on the 14th day, the animals being killed on the 16th day. The spleens and thymuses of all animals were fixed in 4% formaldehyde saline at 4° C. for 4 hr. and imprint preparations for 1 hr. in absolute methyl alcohol. Paraffin sections and imprints were covered in Kodak stripping film (A.R. 50) and exposed for 30 days. Sections treated with hyaluronidase were similarly exposed.

EXTRACTION AND TRANSFUSION OF KURLOFF CELL MATERIAL

The spleens from six female guinea-pigs (weight 600–700 g.) treated with oestradiol benzoate for 18 days were removed under aseptic conditions, placed in a sterile Petri dish and frozen and thawed twice on a freezing microtome. The spleens were then minced and ground with 20 ml. of sterile, buffered saline at pH 7, centrifuged and the supernatant fluid stored at -20°C . Samples of the extract were dried on slides and stained by the PAS and Alcian blue techniques. Three young male guinea-pigs (weight 270–320 g.) were then injected intraperitoneally with the extract on 3 successive days, each pig receiving the material extracted from two spleens. The animals were killed on the 8th, 10th and 12th days after the first injection and the spleen and thymus examined for the presence of Kurloff cells.

RESULTS

Histogenesis of Kurloff cells after administration of oestrogen

The earliest changes in the formation of Kurloff cells are seen 6 days after oestrogen administration. At this time small droplets staining intensely with the periodic Schiff method appear in the cytoplasm of immature lymphoid cells in the thymus (Pl. 1, fig. 1), spleen and lymph nodes. Morphological classification of these cells is difficult at this period, and they resemble 'reticulum cells' or the stem cells normally seen in the lymphoid tissue, and probably representing the precursors of lymphocytes. Maturation of the early forms of Kurloff cells occurs rapidly and the droplets are then seen in the cytoplasm of cells with the nuclear characteristics of small and medium lymphocytes; such cells may be seen in large numbers in the pulp and efferent lymphatics of the thymus (Pl. 1, figs. 2, 3), in the circulating blood,

in the splenic pulp and bone marrow, and are present in smaller numbers in the medulla and sinuses of the lymph nodes. As the Kurloff cell develops, the diameter of the cytoplasmic inclusion body increases in a period of 12–14 days from a minimum of $1\ \mu$ to a maximum of $15\text{--}20\ \mu$, and is surrounded by a thin layer of strongly pyroninophilic cytoplasm. There is little evidence of cellular proliferation during this reaction; few mitoses are seen; and a considerable formation of Kurloff cells occurs in the thymus simultaneously with an acute involution of the organ, resembling the thymic involution produced by oestrogens in other species.

The reaction appears to take place mainly by conversion of a proportion of the lymphoid cells present at the time of the hormonal stimulus and therefore, apart from the formation of the inclusion body, is not detectable by histological examination. The total quantity of material formed in the body is remarkable. Nadel (1952–3) has shown that the weight of the spleen may be doubled from Kurloff cell infiltration after oestrogenic stimulation lasting several weeks. This result indicates the presence of about 0.5 g. of the inclusion substance in the spleen alone. As, in addition, large numbers of Kurloff cells are present in the thymus, peripheral blood, bone marrow and in the capillaries of the lung, it is possible that a total of several grammes of the material may be formed in an animal at the height of the reaction.

The fate of the Kurloff cell is uncertain, with prolonged stimulation the size of the spleen and the number of cells in the peripheral blood become stationary and the continued production of fresh cells is apparently balanced by the loss of earlier generations.

In animals given a single injection of oestrogen and killed at periods up to 35 days later, it was observed that reduction in the number of Kurloff cells in the spleen took place after 30 days and was accompanied by considerable variation in the size of the inclusion droplets.

Transfusion of Kurloff cell material

Saline extracts prepared from the spleens of oestrogen-treated animals containing large numbers of Kurloff cells gave intense staining with PAS and Alcian blue after drying on slides, and a considerable quantity of similar staining material could be precipitated from the solution by addition of alcohol to a 80% concentration. Injection of this extract in young guinea-pigs, however, caused no production of Kurloff cells and no lesions in the animals apart from the formation of PAS positive droplets in the walls of some arteries and of deposition of similar staining material as casts in the renal tubules.

Histochemistry of Kurloff cells

The main histochemical reactions of the inclusion body of the Kurloff cell are given in Table 1. The reactions of the inclusion body can be divided into three groups:

- (1) Reactions indicating the presence of protein.
- (2) Reactions indicating carbohydrate material with 1, 2 glycol groups.
- (3) Reactions indicating the presence of a sulphated mucopolysaccharide.

The first two groups of reactions can be elicited from material fixed in formaldehyde-saline at room temperature. The third group could not be obtained satis-

factorily after fixation at room temperature with any water-containing fixative employed, partial or nearly complete loss of the material taking place. Satisfactory results were obtained by wet-fixation of imprint preparations in Carnoy's fluid or absolute methyl alcohol, or by fixation of tissue in 4% formaldehyde saline at 4° C. for 4 hours. Even at low temperature, loss of mucopolysaccharide occurred if fixation was prolonged beyond this time and was indicated by progressive loss of the intense Alcian blue reaction (Pl. 2, fig. 8) to a thin rim surrounding the droplet, and by the appearance in the fixative of a material precipitable by alcohol and giving strong Alcian blue staining. Satisfactory results with the methylene-blue extinction method were only obtained on imprint preparations. In tissues given short formalin fixation, complete loss of the inclusion tended to occur in the lower pH ranges.

Table 1. *Main histochemical reactions of Kurloff cells*

Millon reaction	+	.
Sakaguchi reaction	+	.
Adams (1957) reaction for tryptophane	±	Weak positive only in sections.
PAS reaction	+	(Pl. 2, fig. 7).
PAS after diastase	+	.
PAS after hyaluronidase	+	.
Alcian blue at pH 0.2	+	Maximal in imprint preparations (Pl. 2, fig. 8)
Alcian blue after hyaluronidase	—	.
Performic-acid Alcian Blue	—	.
Toluidine blue for metachromasia	+	Alcohol resistant in imprints (Pl. 2, fig. 9)
Toluidine blue after ribonuclease	+	.
Toluidine blue after hyaluronidase	—	.
Ferric ferricyanide method for reducing groups	—	.
Methyl green pyronin	+	In cytoplasm surrounding inclusion body
Methyl green pyronin after ribonuclease	—	.
Sudan black after formol-calcium	—	.
Baker's acid haematin method	—	.
Methylene blue extinction point	Below pH 2.6	In imprint preparations fixed in absolute methyl alcohol

The results of autoradiography on oestrogen-treated animals treated with ³⁵S (Pl. 1, fig. 5) indicated a marked uptake of the isotope, forming a pattern in the splenic pulp corresponding to that in a parallel section stained with Alcian blue (Pl. 1, fig. 4). Control animals given ³⁵S showed a diffuse uptake only (Pl. 1, fig. 6), as did sections from treated animals exposed to hyaluronidase before autoradiography.

These histochemical reactions of the Kurloff cell and its utilisation of ³⁵S have accordingly a resemblance to those of the matrix of cartilage or the ground substance of connective tissue.

Material containing numbers of Russell bodies was employed concurrently with Kurloff cells in some of these investigations. Russell bodies give a similar intense PAS reaction to Kurloff cells, but show no evidence of containing mucopolysaccharide material.

DISCUSSION

The interpretation of the results given here is of considerable difficulty. The formation of a material resembling cartilage matrix or connective-tissue ground-substance represents an unknown function of normal lymphoid tissue and no precedents exist

for judging its significance. In addition, this formation apparently takes place in the cytoplasm of lymphocytes, cells whose normal function is also unknown. As a response to oestrogens, however, the formation of such material is not entirely unique as the 'sex skin' of macaque monkeys may show considerable accumulation of mucopolysaccharide following oestrogen treatment.

Some authors (Nadel, 1952-3) have suggested that the Kurloff cell inclusion body represents storage of material derived possibly from the connective tissues. From the results given here this might represent storage of mucoprotein and mucopolysaccharide removed from the connective tissue or cartilage of the treated animal. Such an interpretation is unlikely, however. The Kurloff cell is not of the reticulo-endothelial type normally associated with storage; the injection of the extracted inclusion material does not produce Kurloff cells; and the high concentration of ribose nucleic acid in the cytoplasm of the Kurloff cell suggests protein synthesis rather than storage. The secretory theory, suggested by many workers, is more in keeping with these facts. If secretion occurs, the inclusion material must either pass through the cell wall or rupture of the cell must occur. As the inclusion material is highly soluble in saline or plasma it is unlikely that any direct observation of this is possible, but the irregular size of the inclusion bodies in the last stages of the Kurloff cell reaction is compatible with progressive passage of the material through the cell wall.

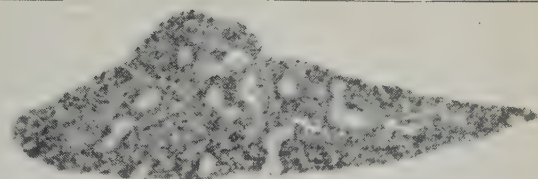
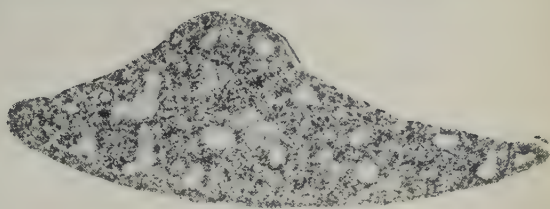
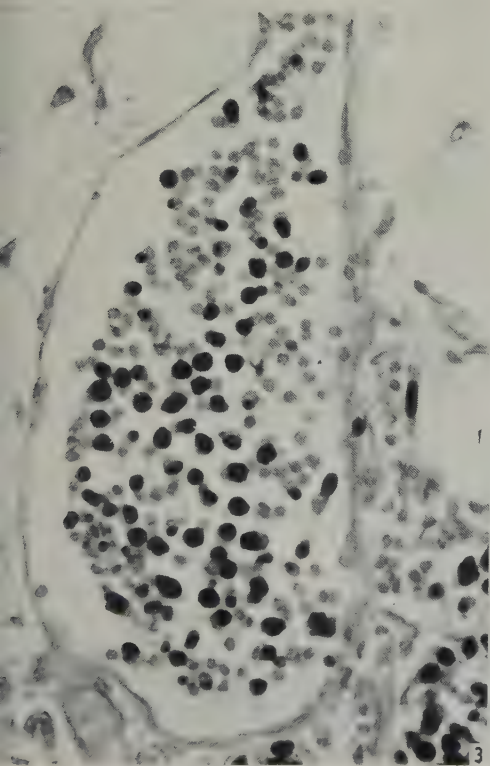
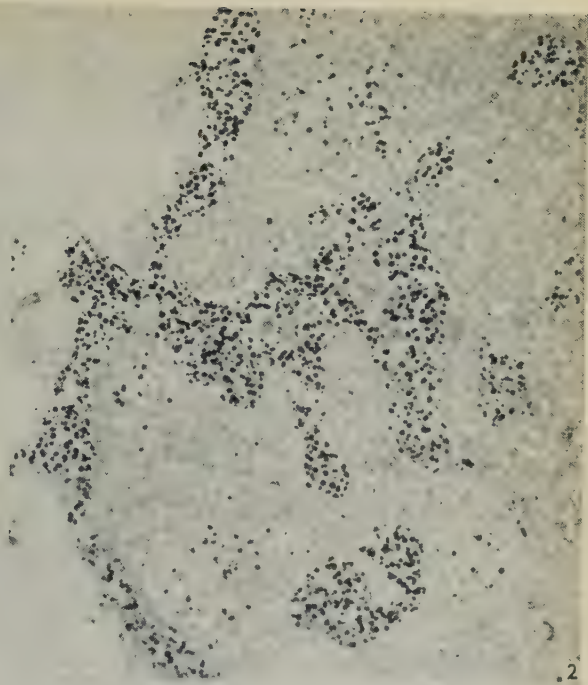
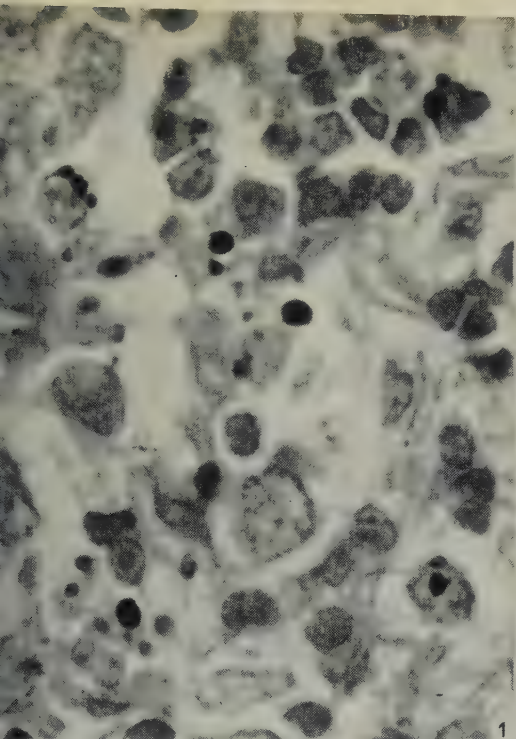
The physiological significance of the secretion of material of this type is obscure, but the large quantities formed suggest direct participation in some growth process. The formation of Kurloff cells in the normal animal reaches its peak during pregnancy and, in view of the probable life span of the cells, any release of inclusion material would reach its maximum in the second half of gestation when the greatest quantity of foetal connective tissue and cartilage is formed. The possible passage of Kurloff cell material into the foetus or placenta requires further investigation and might be undertaken by the use of a radioactive tracer.

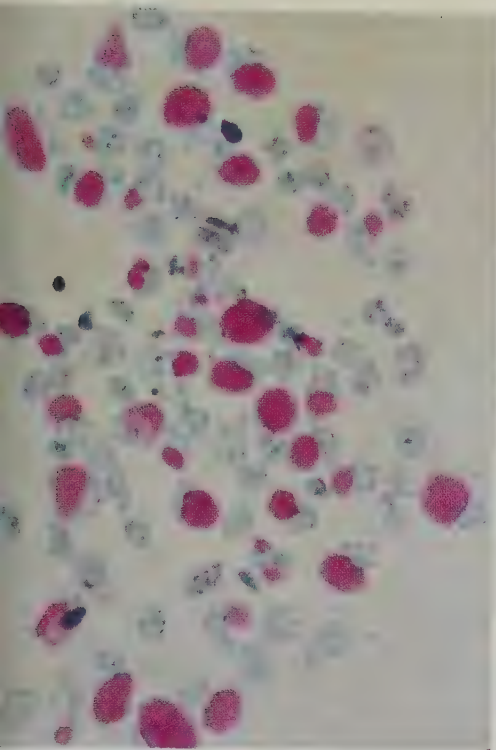
It was stated earlier that formation of Kurloff cells was confined to the guinea-pig. The significance of this, however, is uncertain as the reaction is detectable only by the formation of the cytoplasmic inclusion. If formation and release of similar material took place in other species it would be detectable probably only by chemical methods. The description by Shetlar, Kelly, Foster, Shetlar & Everett (1950) and Kuhns & Hyland (1956) of the high serum levels of mucoprotein and glucosamine polysaccharide in the second half of human pregnancy is of interest in this connexion.

SUMMARY

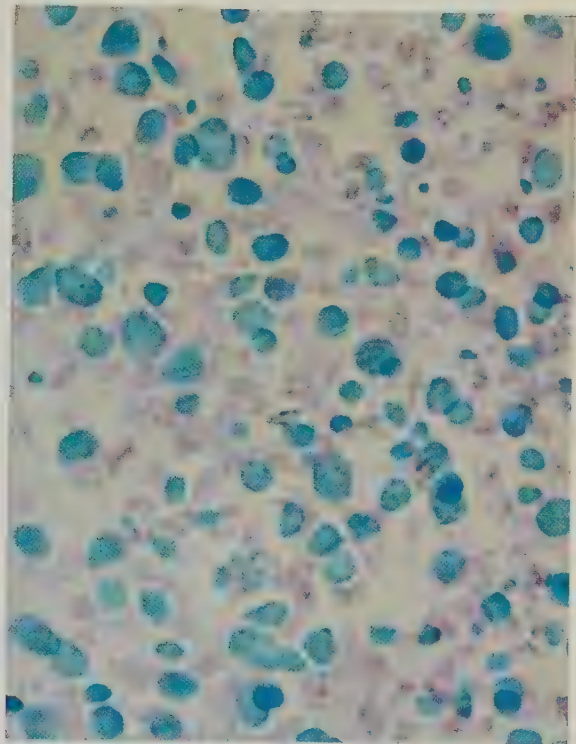
A histochemical investigation has been made of the composition of the inclusion body of the Kurloff cell. It is concluded that this is composed of a complex containing muco- or glycoprotein and a sulphated mucopolysaccharide, and is related to the ground substance of connective tissue or cartilage. The significance of the secretion of material of this type in the pregnant animal is discussed.

We are indebted to Prof. D. S. Russell and Prof. R. J. Harrison for much help and encouragement in the preparation of this paper and to Dr C. W. M. Adams for advice on technical methods.

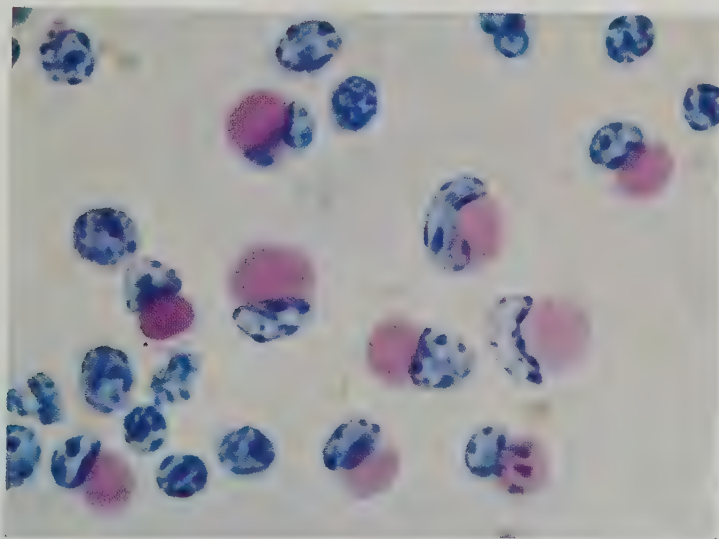




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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Formation of inclusion material in immature lymphoid cells of thymus 8 days after administration of oestrogen. PAS method. $\times 1340$.
- Fig. 2. Formation of Kurloff cells in cortex of thymus. PAS method. $\times 115$.
- Fig. 3. Large number of Kurloff cells in efferent lymphatic of thymus. PAS method. $\times 450$.
- Fig. 4. $10\ \mu$ section of spleen to show pattern in red pulp produced by Kurloff cell infiltration. Alcian blue. $\times 5$.
- Fig. 5. Autoradiograph following ^{35}S injection, of same spleen as Fig. 4, to show similar pattern in red pulp. $\times 5$.
- Fig. 6. Autoradiograph of spleen of control animal containing no Kurloff cells, showing diffuse uptake of ^{35}S only. $\times 5$.

PLATE 2

- Fig. 7. Mature Kurloff cells from splenic pulp. Imprint preparation fixed in Susa's fixative. PAS method. $\times 640$.
- Fig. 8. Kurloff cells from splenic pulp. Alcian blue at pH 0.2. Imprint preparation fixed in Carnoy's fluid. $\times 550$.
- Fig. 9. Kurloff cells from splenic pulp to show metachromasia of inclusion body. 1% Toluidine blue. Fixation in absolute methyl alcohol. $\times 1100$.

THE HISTOCHEMICAL LOCALIZATION OF SPECIFIC CHOLINESTERASE IN THE LYMPHATIC TISSUE OF MAMMALS

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Dale (1914) first put forward the hypothesis that the transient activity of acetylcholine *in vivo* was probably due to its destruction by an esterase contained in the blood. In 1926 Loewi & Navratil demonstrated that such an enzyme was present also in tissue extracts. Further investigations in this field led to the recognition of different types of cholinesterases, extractable both from different tissues of the same animal and from animals of different species. These esterases were classified into two main types: acetylcholinesterase or specific cholinesterase, and pseudocholinesterase. Both types are present in numerous organs and tissues of mammals (Ord & Thompson, 1950). Each of these enzymes can hydrolyse other cholinesters in addition to acetylcholine. Ord & Thompson (1950), in a study on the distribution of these enzymes in the rat, came to the conclusion that there are tissues which contain specific cholinesterase in large amounts (brain, striated muscles, adrenal glands), others which contain both acetylcholinesterase and pseudocholinesterase in almost equivalent amounts, and finally tissues containing mostly pseudocholinesterase.

Methods suitable for demonstrating the precise localization of this enzymic activity from a cytological point of view in the individual components of various tissues have been introduced (Gomori, 1948; Koelle & Friedenwald, 1949). Investigation of lymphatic tissue with these techniques has till now received little attention. Only the thymus, which has an association with this tissue, has been studied by Rogister, Dumoulin & Gerebtzoff (1955). The results obtained by these investigators, using the method of Koelle & Friedenwald as modified by Gerebtzoff, demonstrated a slight and temporary acetylcholinesterase activity in some epithelioid elements of the medulla of this organ and in Hassall's cells and corpuscles. This finding partly confirmed the quantitative data published by Glick, Lewin & Antopol (1939), who with a biochemical method studied the cholinesterase activity of extracts of numerous organs in swine, including the thymus, the spleen and the mesenteric and supracervical lymph nodes. The histological localization of cholinesterases in lymph nodes, palatine tonsil and spleen, as principal organs of the lymphatic system, form the subject of this study. Its purpose has been to assess whether the enzymic activity, reported by Glick *et al.* (1939) in these organs, is related to intense functional activity of the lymphatic follicle.

Since this study was completed (1956) we have found a communication to the Biological Society of Lyon by Dumont (1955) on the histochemical localization of acetylcholinesterase in the spleen and lymph nodes of small mammals. More

recently, Rogister and Gerebtzoff (1958), in a review of histochemical investigations on cholinesterases, take into consideration the lymphatic organs. These last two contributions will be discussed later.

MATERIAL AND METHODS

The relevant organs were removed from a dozen young and adult cats, which are known to possess a well-developed lymphatic system. In each animal preparations of palatine tonsil, lymph nodes of different regions and spleen were made. Lymph nodes and spleen of other small mammals, such as rat and rabbit, were also examined. The thiocholine method of Koelle & Friedenwald (1949) modified by Gerebtzoff (1953) was used. It will be sufficient here to summarize the technique. Frozen sections, 25–50 μ thick, of organs, fixed in 10 % neutral formol for a period of 5–8 hr., were incubated in a substrate of known buffered pH, containing acetylthiocholine for the demonstration of acetylcholinesterase, or butyrylthiocholine for the demonstration of pseudocholinesterase. To assess whether a positive reaction was really due to the presence of acetylcholinesterase, sections were previously incubated for 30 min. in a 10^{-7} M solution of di-isopropylfluorophosphate (DFP), which selectively inhibits the pseudocholinesterases. The thiocholine liberated during the incubation period by hydrolysis caused by the enzyme contained in the tissue, was converted *in situ* into a mercaptide which precipitates as copper sulphide in the presence of ammonium sulphide. The black precipitate of copper sulphide in the tissues localizes the cholinesterases. Incubation was carried out at different pH, varying from 6.2 to 6.8 and for different periods of time. Further details of the technique are to be found in Gerebtzoff's paper (1953). Sections were also stained by usual routine methods.

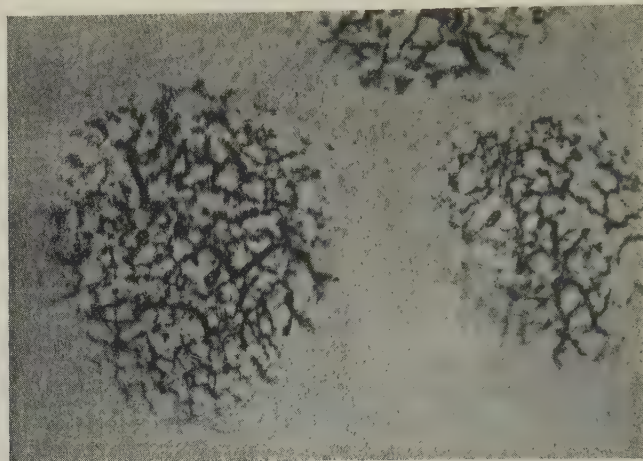
RESULTS

Lymph nodes. Our results are based on the study of sections of lymph nodes of cat, rabbit and rat. On examining the treated sections at low magnification it appears at once that circumscribed areas of lymphatic tissue exhibit a strongly positive reaction. Closer examination shows that this is associated with the germinal centres of lymphatic follicles. The copper sulphide precipitate is mainly located on the walls of small blood vessels which supply the follicle; the most common appearance is that of a thick network, more or less intensely brown stained, which traces the disposition and distribution of vessels in the germinal centre just as demonstrated by indian-ink injections. At the margin of the germinal centre such a positive reaction suddenly ceases. Cells, mostly lymphocytes, surrounding and in contact with the walls of the vessels, also exhibit cholinesterase activity. These appearances were observed in sections incubated with a substrate containing acetylthiocholine and also previously treated with DFP which suggests a specific cholinesterase activity.

Since pretreatment of sections with DFP does not sensibly alter the enzymic staining and the incubation of sections with substrate containing butyrylthiocholine does not produce any staining, it would seem that pseudocholinesterase is absent in these organs.

It is noteworthy that in a single section the intensity of the enzymic staining can vary considerably from follicle to follicle and that some germinal centres may even

be unstained. In no other zone or structure of the lymph node is there evidence of cholinesterase activity. However, the walls of small vessels coursing on the border of the follicles and of venous sinuses show sometimes a weak esterase activity. The small (primary) follicles without lighter central areas and the medullary cords do not exhibit any cholinesterase activity.



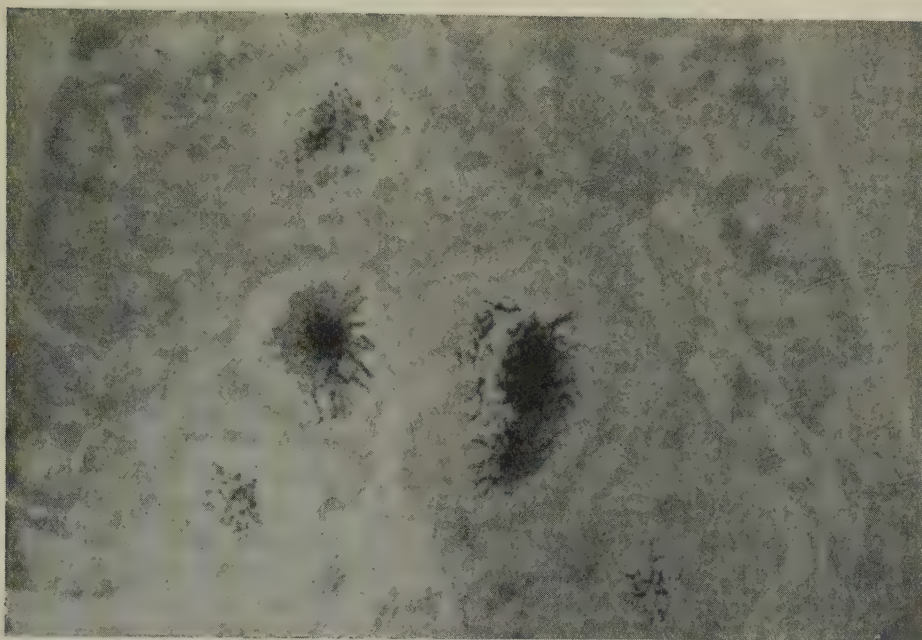
Text-fig. 1. Rabbit lymph node: the black precipitate of copper sulphide impregnating the vascular network of germinal centres demonstrates the high concentration of acetylcholinesterase. Section, 30 μ . Incubation for 90 min. in acetylthiocholine substrate at 6.8 pH, after treatment with DFP. $\times 135$.

Palatine tonsil. Our observations were carried out on preparations of palatine tonsils of young and adult cats and rabbits. In this organ also the enzymic activity is restricted to the germinal, or reaction, centre of follicles. The reaction is particularly intense in the small vessels supplying the central area. The walls of capillaries are stained a dark brown and the cellular elements which surround them as a sheet are intensely positive. Where vessels are cut longitudinally, their lumina are outlined by two black parallel lines corresponding to the endothelial cells and to a series of lymphoid and histiocytic elements also exhibiting acetylcholinesterase activity. The tonsil usually possesses a large number of lymphatic follicles with intensely positive germinal centres. Unlike the lymph nodes, no tonsillar follicles were found without signs of esterase activity or primary follicle (Pl. 1).

Spleen. The cholinesterase activity of the spleen is also localized in the lymphatic follicles and more precisely in their vascular network and a number of the lymphoid cells surrounding the vascular wall. The positivity of the reaction seems, however, to be weaker in the vessels here than in those of lymph nodes and palatine tonsil. Unlike these organs, where only cells in contact with vessels present a positive reaction, in the spleen a positive reaction extends to a larger number of lymphoid and histiocytic elements.

Attempts to demonstrate a pseudocholinesterase activity in the spleen by incubating sections with substrate containing butyrylthiocholine were unsuccessful;

it would therefore appear that in the spleen, too, only acetylcholinesterase activity is present.



Text-fig. 2. Rat spleen: germinal centres of Malpighian follicles exhibiting a different degree of enzymic staining. In this organ the lymphatic cells, positive to the reaction, are more numerous and they mask the outline of blood capillaries. Section 30μ . Incubation 90 min. in substrate containing acetylthiocholine at pH 6.8, after prior treatment with DFP. $\times 60$.

DISCUSSION

A critical analysis of the method used in this study is beyond our present aim. To assess its validity and specificity the reader is referred to the original papers of Koelle & Friedenwald (1949), Koelle (1951) and Gerebtzoff (1953). In spite of certain discrepancies and although further improvements are desirable, application of the technique seems justified and acceptable in the light of the positive data already acquired and after the modifications of the original method made by Koelle himself (1951) and Gerebtzoff (1953). The original pitfalls of the method, such as enzymic diffusion and incomplete inhibition of pseudocholinesterases, have been remarkably reduced. Furthermore, this technique seems to be adequate from the purely chemical point of view (Malmgren & Sylvén, 1955).

In our study the spleen, the palatine tonsil and lymph nodes of different regions of cat and rabbit, and spleen and lymph nodes of rat were systematically examined, and it was found that in these organs the acetylcholinesterase activity is localized in the germinal centres of lymphatic follicles and more precisely in the wall of their blood capillaries. This enzymic activity often extends to the lymphoid or histiocytic elements situated in intimate contact with the walls of these capillaries. Moreover, in the various organs studied, it is possible to observe in the same section lymphatic

follicles exhibiting different degrees of enzymic staining, so that near follicles with a strongly positive reaction there are others showing weak or negative reaction. The sections of spleen studied with this method differ from those of lymph nodes and palatine tonsils, for the intensity of the enzymic staining of Malpighian follicles is more variable than in the other organs and a larger number of the splenic perivascular cells react positively. Observation and comparison of numerous sections leads us to believe that the site of acetylcholinesterase activity is in the wall of capillaries of the germinal centre of lymphatic follicles, and that the perivascular cells probably owe their positivity to enzymic diffusion.

It is known (Rogister & Gerebtzoff, 1958) that in the spleen of some animal species, as, for example, cat and rabbit, the megakaryocytes demonstrate acetylcholinesterase activity. However, it does not seem that the perivascular cells with positive reaction in this organ are identifiable as megakaryocytes either by their morphology or dimensions. The acetylcholinesterase activity of germinal centres of spleen must be considered as analogous to that of germinal centres of tonsil and lymph nodes.

The validity of these observations is supported by the constancy of the appearances in lymphatic organs of animals of the same and of different species and by controlling the reaction with the simultaneous incubation of sections of striated muscular tissue. Furthermore, the findings are supported by the negative results obtained after incubation of sections of lymphatic tissue with butyrylthiocholine, which reveals the presence of pseudocholinesterases. Further pre-treatment of sections with a dilute solution of DFP, specific inhibitor of pseudocholinesterases, only interfered slightly with the enzymic hydrolysis of the acetylthiocholine. The results confirm and extend those obtained with quantitative methods by Glick *et al.* (1939) in the lymphatic tissue of swine—and define the cyto-histological localization of the enzyme.

Dumont (1955) has published results which do not agree with ours. He observed the presence of cholinesterase in a large number of structures belonging to lymphatic organs of small mammals. His illustrations show evidence of enzymic diffusion, and we consider that some of his positive results are due to the use of an insufficiently specific method. We are unable to confirm fully some findings by Rogister & Gerebtzoff (1958) on the presence of true and pseudocholinesterases in the smooth muscle fibres of splenic trabeculae, on the pseudo-enzymic staining of the red pulp due to deposits of hemosiderin and on a positive reaction in the venous sinuses of lymph nodes.

The discrepancies between our results and those of the above-mentioned are possibly due to the different periods of incubation used. We found that the optimum time for clear enzymic staining, without obvious enzymic diffusion, was approximately 90 min. A quantitative and comparative evaluation of cholinesterase activity in organs of different animals, on the basis of intensity of the staining, seems very difficult if not impossible, for this intensity is affected by slight variations in the time of incubation.

An acceptable explanation of the functional significance of acetylcholinesterase in the vascular walls of lymphatic follicles has not yet been advanced. The explanation given by Rogister & Gerebtzoff (1958), that the slowing down of the blood circulation in the capillary network of lymphatic follicles is the cause of the enzymic

deposit in the vascular walls, is not very convincing since there are other capillary networks in which slowing down of the blood flow occurs without evidence of acetylcholinesterase activity.

Lymphatic follicles with germinal centre or secondary follicles are generally regarded as transient structures, and indeed various authors, including Dabelow (1936), Muratori (1938) and Hellmann (1943), agree on their origin from primary follicles with uniform capillary network and structure. The secondary follicles, whose capillary network is dense in the peripheral dark-staining zone, and loose in the central pale-staining zone, become changed, according to Fischer (1937) into tertiary nodules or the pseudosecondary nodules of Ehrlich, and later into lymphoreticular tissue. It should be stressed that only the secondary follicle possesses a capillary network exhibiting intense acetylcholinesterase activity which is histochemically detectable. On the other hand, the particular reactivity of this capillary bed to toxic stimuli (Mottura, 1951) can perhaps be related to the amount of acetylcholinesterase in the vascular walls. Moreover, it is possible that the variable intensity of the enzymic staining of germinal centres of lymphatic follicles reflects different stages in their functional activity. The fact that, in tissue culture, choline induces transformation of fibroblasts or of muscle fibres into macrophages (Chèvremont, 1949) leads, by analogy, to the hypothesis that the proliferation and differentiation of cells in the germinal centre of lymphatic follicles might be related to the presence and metabolism of acetylcholine in the capillary vessels of this structure.

SUMMARY

The histochemical localization of specific cholinesterase in the lymphatic tissue of small mammals (cat, rabbit and rat) was studied by means of the 'thiocholine method' of Koelle and Friedenwald, modified by Gerebtzoff. In the palatine tonsil, lymph nodes of different regions and the spleen, the acetylcholinesterase activity is localized to the wall of blood capillaries supplying the germinal centres of lymphatic follicles. Lymphoid or histiocytic elements in intimate contact with the wall of these vessels also show a positive reaction. Only secondary follicles possess a capillary network in which intense acetylcholinesterase activity is histochemically detectable. Lymphatic follicles can exhibit different degrees of enzymic staining. The possible functional significance of these findings is discussed.

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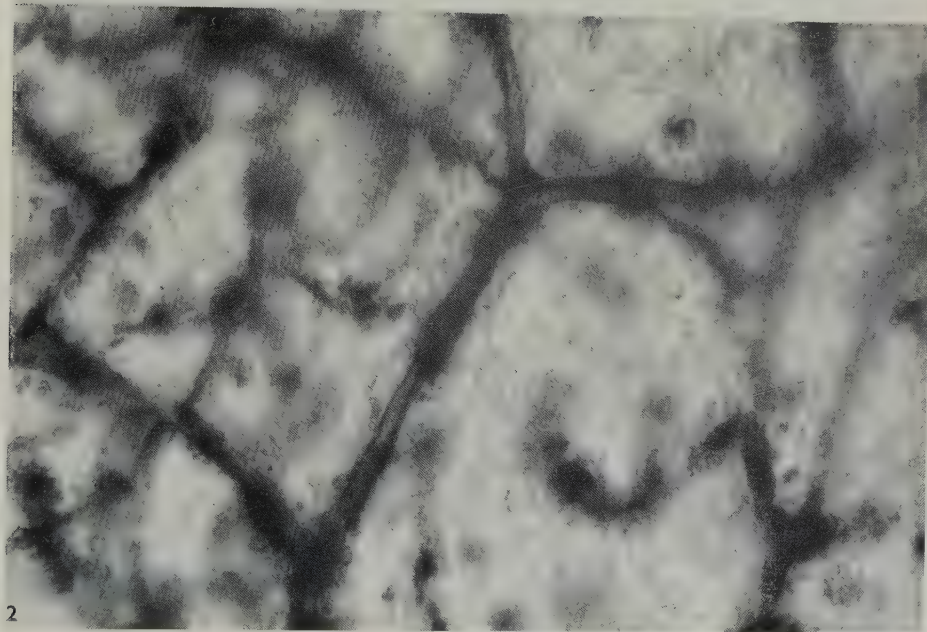
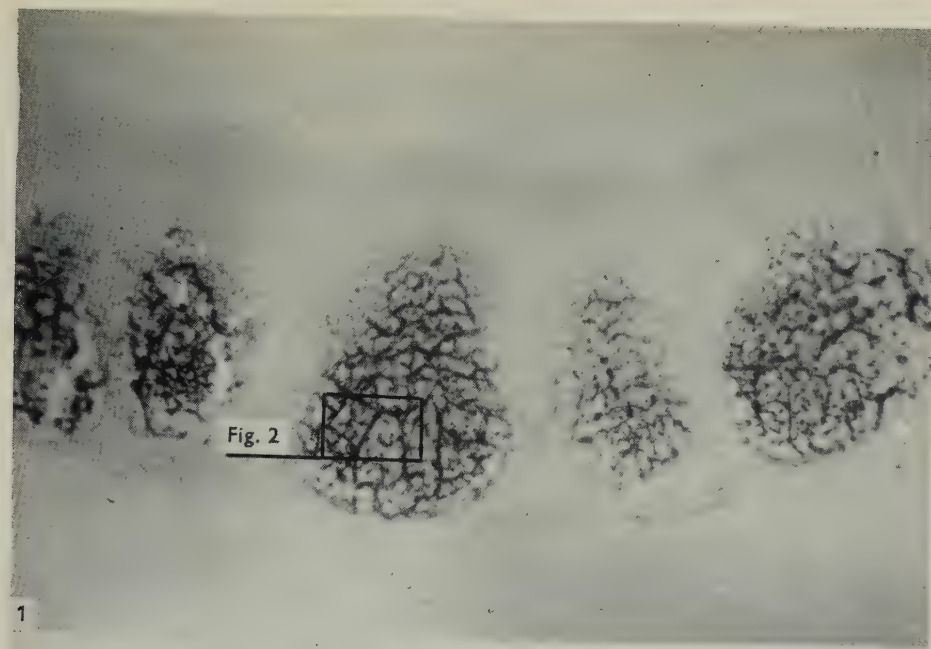
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EXPLANATION OF PLATE

Fig. 1. Palatine tonsil of cat: the walls of blood capillaries of germinal centres are stained black by precipitates of copper sulphide. Section, 30μ thick, incubated for 90 min. in substrate containing acetylthiocholine, after previous inhibition with DFP, pH 6 and 8. $\times 60$.

Fig. 2. Higher magnification of area outline in fig. 1. The acetylcholinesterase activity is clearly localized on the walls of blood capillaries. Around these there are several lymphatic cells exhibiting a positive reaction. $\times 620$.



D'AGOSTINI AND ROSSATTI—SPECIFIC CHOLINESTERASE IN THE LYMPHATIC TISSUE OF MAMMALS

(Facing p. 360)

POSTNATAL CHANGES IN THE HISTOLOGY OF THE SEMINAL VESICLE AND COAGULATING GLAND IN THE RAT

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INTRODUCTION

Although several studies have been made of the prenatal development of the accessory sexual organs of the rat (Pallin, 1901; Wiesner, 1934; Price, 1936), little attention has been paid to changes occurring before and at puberty. Price (1936) studied all the accessory organs up to the fifth day of life, but at later stages she described in detail only the changes in the ventral prostate. Wiesner (1934) confined his attention mainly to the dimensions and weights of the seminal vesicle.

During an investigation into the age changes in fructose content of the rat coagulating gland (Clegg, unpublished work), degenerative changes were noted in the organs of rats sacrificed at the age of 21 days, and these findings were repeated in animals of greater age, although they became less marked as the animal approached puberty. Similar changes were also seen in the seminal vesicles. It was decided to extend this part of the investigation to include animals from birth until puberty—that is, the age at which sperm heads were observed in the seminiferous tubules. This proved to be about 40 days.

MATERIAL AND METHODS

Twenty-one albino rats, obtained from three independent sources, were used in the investigation. The ages of the animals varied from one to 55 days.

Using ether anaesthesia the left seminal vesicle, with its accompanying coagulating gland, and the left testis, were removed. The organs were fixed in Bouin's fluid and sections 8μ thick were cut and stained with Ehrlich's haematoxylin and eosin.

RESULTS

One day (one animal)

The anlage of the seminal vesicle lies dorsolateral to the mesonephric ducts. It possesses a lumen, and the average height of its cells is 17μ .

The anlage of the coagulating gland is present as a solid rod of cells ventrolateral to the mesonephric and paramesonephric ducts. It penetrates the mesenchyme to only a short distance from the urogenital sinus. Its diameter averages 35μ . In both organs mitoses are numerous.

Three days (one animal)

No marked alteration is observed from the pattern 2 days previously. The anlage of the seminal vesicle contains a little structureless eosinophilic material. That of the coagulating gland has penetrated further in a dorsolateral direction, and its free

end has expanded somewhat. Its average diameter has increased to 45μ . There are numerous mitoses in both organs.

Five days (one animal) (Pl. 1, fig. 1)

Little change has occurred in the seminal vesicle. In the coagulating gland, dorsolateral extension has continued and the diameter has increased to 75μ . A lumen is visible in that part destined to become the duct of the gland. Mitoses are numerous.

Seven days (one animal)

Seminal vesicle

Extensions from the central lumen of the organ are now burrowing into the mesenchymal sheath. The average height of the epithelium has increased to 20μ , and mitoses are frequently seen.

Coagulating gland

The diameter remains approximately the same, and the process of canalization is extending towards the secretory part of the gland.

Ten days (one animal) (Pl. 1, fig. 2)

Seminal vesicle

This can now be distinguished as a projection into the peritoneal cavity. Instead of a simple sac-like appearance, it has a central lumen with acinar extensions. The cells are columnar, with a large basal nucleus, and slightly eosinophilic cytoplasm; they have an average height of 18μ .

Coagulating gland

By now this is a compound acinar structure, contained within the same serous and connective tissue sheath as the seminal vesicle. The acini are not yet canalized and average 38μ in diameter. Mitoses are frequent.

Seventeen days (one animal)

Seminal vesicle

The acini have increased in number and size. The cells are columnar, with an average height of 14μ and a moderately eosinophilic cytoplasm. There is a small amount of eosinophilic secretion.

Coagulating gland

About half the acini are now patent. Their diameters vary between 40 and 60μ and they are usually lined by single layers of cells, whose average height is 11μ . There are many mitoses.

Most acini contain a structureless substance, which is rather more eosinophilic than the cytoplasm of the epithelial cells. A few acini contain collections of necrotic epithelial cells.

Twenty-one days (one animal)

Seminal vesicle (Pl. 1, fig. 3)

The epithelial cells are columnar, with a large, oval, basal nucleus and slightly eosinophilic cytoplasm. The cell height averages 14μ . Occasional masses of eosinophilic secretion are present.

The distal central lumen of the gland contains quantities of epithelial debris, and over considerable areas the walls are denuded of epithelium. In some regions karyopyknosis of epithelial cells may be observed in the walls of the organ. In areas where denudation or karyopyknosis has occurred, masses of structureless eosinophilic material may be seen, apparently adhering to the wall of the vesicle. This material is indistinguishable from the secretion noted above, except that it is not separated from the wall of the organ by a clear space. In several instances there are collections of similar structureless material deep to the epithelium, and where the epithelium has become denuded these collections are continuous with the luminal masses.

Coagulating gland (Pl. 1, fig. 4)

About two-thirds of the acini are patent, and occasional convolutions of the lining epithelium may be seen. The cell height is rather variable, averaging 15μ . The acinar diameters vary between 40 and 60μ . Most acini are lined by a single layer of cells; occasionally a second layer may be seen. The nuclei are vesicular and basal, with a markedly eosinophilic cytoplasm. Mitoses are frequent.

About a quarter of the acini contain debris consisting of aggregations of cells with deeply basophilic homogeneous nuclei and very eosinophilic cytoplasm. Cells possessing similar characteristics may be seen in the walls of some acini, where they stand out prominently by comparison with their neighbouring, less deeply stained cells.

Twenty-eight to thirty days (five animals)

Seminal vesicle (Pl. 2, fig. 5)

The cells are slightly lower than at previous ages, averaging 11μ in height. Their histological characteristics have not otherwise altered.

The degenerative changes do not differ materially from those seen at 21 days, except that the areas of degeneration are more extensive, and there is a greater accumulation of cellular debris in the lumina. In some regions whole acini appear to be completely disorganized and in such areas the subepithelial tissue of the wall of the organ appears to be infiltrated by the same structureless eosinophilic material noted at 21 days. In these regions there is no discontinuity between material in the wall and in the lumen.

Coagulating gland (Pl. 2, fig. 6)

All the acini are patent. They average 90μ in diameter, and the cell heights 14μ . The cells differ little in appearance from those seen at 21 days.

In addition to necrotic cells in the acinar walls and cellular debris in lumina, areas of epithelium may be seen to be shed into acini. This is only an occasional finding, and even then it is localized to only a part of an acinus. In such regions, the wall of the acinus consists of the connective tissue and muscular sheath of the gland, together with the membrana propria. Occasional epithelial cells may remain adherent. Although less generalized, the appearances are similar to those produced by interruption of the arterial supply to the gland (Clegg, 1953, 1954).

*Thirty-five to thirty-seven days (four animals)**Seminal vesicle (Pl. 2, fig. 7)*

Little change has occurred. The degenerated areas are less extensive in three animals when compared with their litter-mates at 28–30 days; in one animal the changes are slightly more extensive.

Coagulating gland

The findings resemble those at the previous age, although degenerative phenomena are not so frequently seen. The acini average 130μ in diameter, and the cell height averages 14μ .

*Forty-one to forty-four days (three animals)**Seminal vesicle (Pl. 2, fig. 8)*

The cell height averages 12μ . The nuclei are basal and the cytoplasm slightly eosinophilic. No refractile granules are seen, although an increased eosinophilia may be observed towards the free border of the cell.

Degenerative changes are apparent in a few acini, and they present the usual features. In addition, however, signs of regeneration are present in two animals. These consist of an apparently intact layer of cubical epithelium covering the membrana propria in areas where epithelial destruction is prominent. The nuclei of these cells are smaller and rounder than those of normal seminal-vesicle epithelium, but they show no evidence of pyknosis.

Coagulating gland

The average diameter of the acini is the same as at the previous age, but the epithelium is rather lower, averaging 11μ . Many of the cells show the basal vacuolation characteristic of maturity, and the nucleus lies in the mid-zone of the cell. Degenerative changes of all three types may be seen, but they are less widespread than at previous stages. One section (Pl. 2, fig. 9) shows the separation of a fold of epithelium from the membrana propria by a structureless eosinophilic substance which resembles that seen in the seminal vesicle.

*Forty-eight to fifty-five days (two animals)**Seminal vesicle*

The cell height is 14μ and small amounts of secretion are present in the acini. The central lumen of the gland contains a few shed cells and occasional pyknotic nuclei are seen in the lining epithelium. No epithelial destruction is seen.

Coagulating gland

This presents an appearance of maturity. A few acini contain masses of secretion, which are almost completely acellular.

DISCUSSION

The findings described in this investigation may be said to agree with those of Price (1936) up to the fifth day of life. At greater ages this author did not study the coagulating gland. In the seminal vesicle she described the occurrence of secretory granules 36 days after birth. Moore, Hughes & Gallagher (1930) regarded these

granules as typical of the adult, mature vesicle, and showed that they disappeared within three days of castration. The present investigation was unable to confirm this finding. This discrepancy is surprising, since, by 40 days, the coagulating gland cells had the characteristic basal vacuolation and mid-cell position of the nucleus, which is regarded by Moore, Price & Gallagher (1930) as being characteristic of maturity.

However, as early as the 30th day the lumen of the seminal vesicle could be seen to contain eosinophilic material, indistinguishable from the secretion of an adult organ. This finding contradicts that of Wiesner (1934), who was unable to find evidence of secretory activity before the 35th day. It would seem that the onset of secretory activity in the seminal vesicle is not necessarily correlated with the appearance of typical secretory granules, although the nature of the secretion may well be changed by the latter event.

Degenerative changes in the seminal vesicle and coagulating gland do not appear to have been described previously, although Ortiz, Price, Williams-Ashman & Banks (1956) included a photomicrograph which showed large intraluminal masses in the lateral prostate of a 10-day-old guinea pig. These authors regarded such masses as evidence of secretion, although they contained distinct cellular elements.

The degenerative processes in the two organs occur at similar periods, and their histological characteristics bear a considerable resemblance to one another; the only significant difference is the greater degree of oedema in the seminal vesicle. Since these organs are anatomically contiguous and under developmental influences which are qualitatively the same, it may be assumed that in each organ the changes observed have the same cause or causes.

In the case of the seminal vesicle the immediate cause of the cell deaths is probably the structureless eosinophilic material which accumulates deep to the epithelium, and which would have the effect of isolating epithelial cells from their sources of nutriment. This material has the characteristics of oedema fluid, and the appearance of the submucosal tissue of the seminal vesicle resembles that produced in the vas deferens of the mouse by the administration of oestrogens (Harsh, Overholser & Wells, 1939).

In the coagulating gland, oedema is seen much less frequently, and although it is a possible cause of cell death, other aetiological factors must be considered.

Since the process of canalization of the acini of the gland occurs shortly before cell deaths are observed (between the 10th and 17th days in these investigations), the presence of masses of cells in acini might be explained on this basis, especially since the usual theory of the formation of lumina in solid rods of cells involves the death of cells in the centre of the rod, the peripheral cells surviving. However, three facts militate against such a conclusion:

- (i) Degenerative phenomena are not seen in uncanalized rods of cells.
- (ii) The walls of canalized acini frequently contain degenerate cells.
- (iii) Occasional acini contain a completely disorganized and degenerate epithelium; the walls consist of connective tissue only.

We may conclude, therefore, that degenerative changes in the coagulating gland, as well as in the seminal vesicle, are not connected with the process of lumen formation. Since the changes occur during the prepubertal and pubertal periods, they

must be associated with the structural and functional maturation of the gland. Cell deaths occurring at this time fall into the category of 'histiogenetic degenerations' (Glücksman, 1951), and they may be caused either by a failure or by a change of the differentiating impulse. In the prepubertal male mammal, androgen is the main stimulus to differentiation of the accessory reproductive organs, and failure in its production obviously does not occur at this age.

Androgens are not the only substances capable of influencing the development of the reproductive system; oestrogens may act synergistically with androgens to produce full development of the accessory organs (Burrows, 1945; Emmens & Parkes, 1947). In this co-operative function, androgens stimulate the epithelia and oestrogens the connective tissue.

It is well known that oestrogens may cause water-retention in the reproductive organs, and this fact may account for the oedema observed in the seminal vesicle and to a lesser extent in the coagulating gland. In the latter organ, however, cell deaths may be seen with no concomitant oedema, and the following hypothesis is advanced to meet the observed facts.

With the rapid increase in gonadal hormone production which occurs before and at puberty it is conceivable that temporary variations may arise in the proportions of androgen to oestrogen, with the result that growth of epithelial and connective tissue elements does not occur *pari passu*. Connective tissue overgrowth due to a preponderance of oestrogen may therefore lead to a strangulation of the blood supply to epithelial cells with consequent cell death. The equilibrium between connective tissue and epithelium would only be established when androgen and oestrogen production became stabilized at the time of sexual maturity.

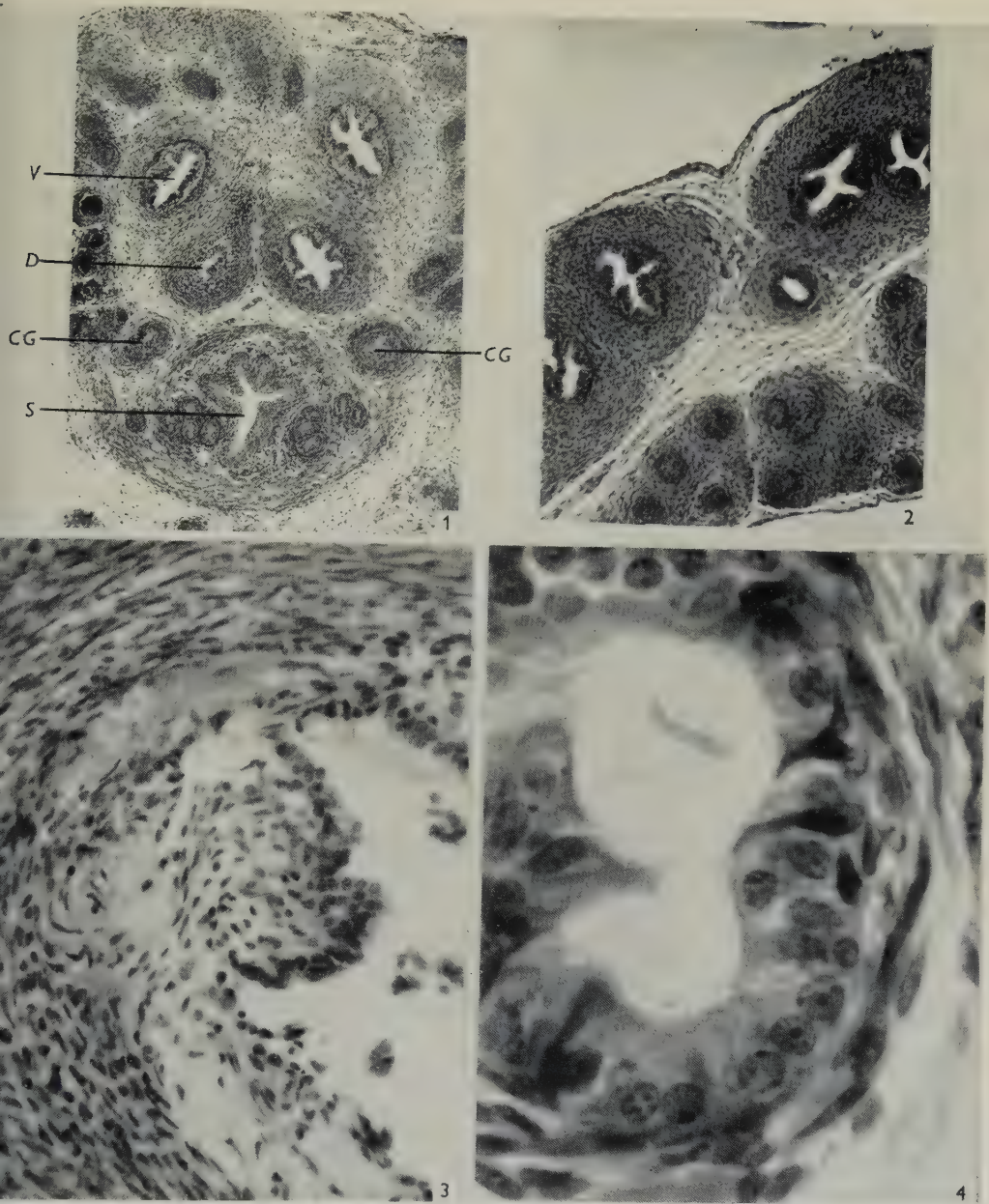
SUMMARY

1. Histological changes in the rat seminal vesicle and coagulating gland have been studied from birth up to an age of 55 days.
2. At 17 days in the coagulating gland, and 21 days in the seminal vesicle, degenerative changes may be seen in epithelia. These are most marked between the 28th and 30th days of life, and thereafter they diminish in intensity. The changes comprise (i) subepithelial oedema, (ii) death of cells in the epithelial walls of the organs, (iii) shedding of cells into the lumina, and (iv) aggregation of these shed cells into compact masses.
3. These appearances are not associated with the process of lumen formation in either organ.
4. It is tentatively suggested that imbalances in the androgen : oestrogen ratio in the growing animal may be responsible for the cell deaths observed.

I am indebted to Prof. R. G. Harrison for his interest and advice. The histological preparations were made by Mrs C. Morley and Miss H. Ireland and the photomicrographs by Mr L. G. Cooper and Mr A. F. Taunton; to all of these I am grateful.

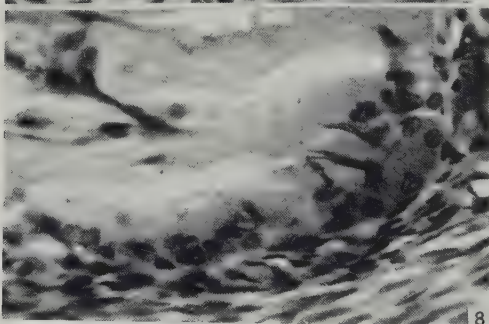
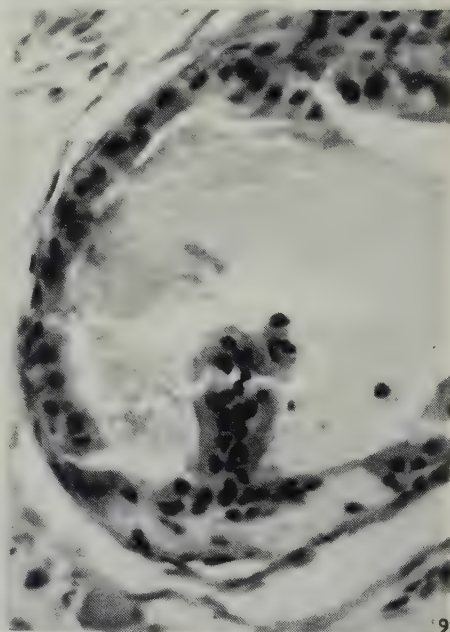
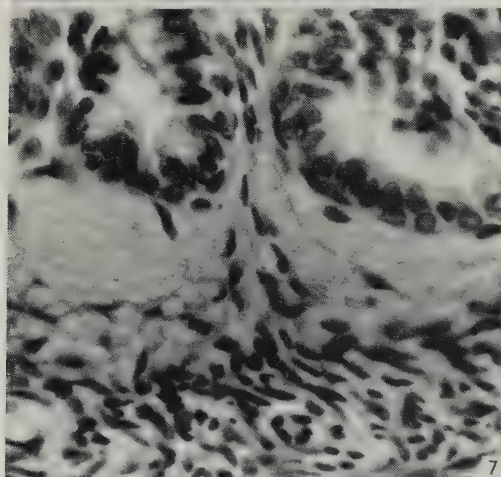
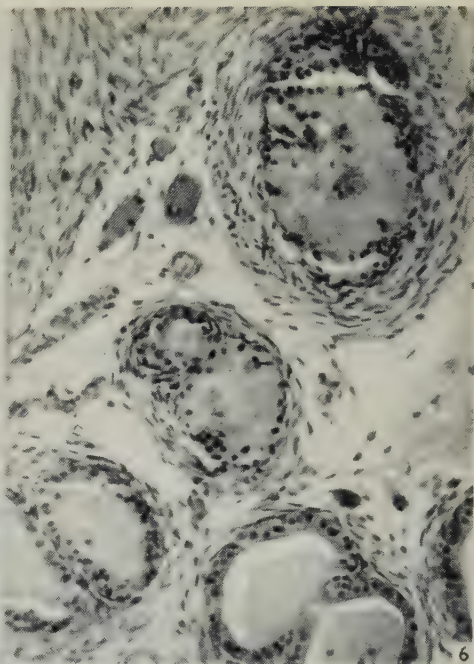
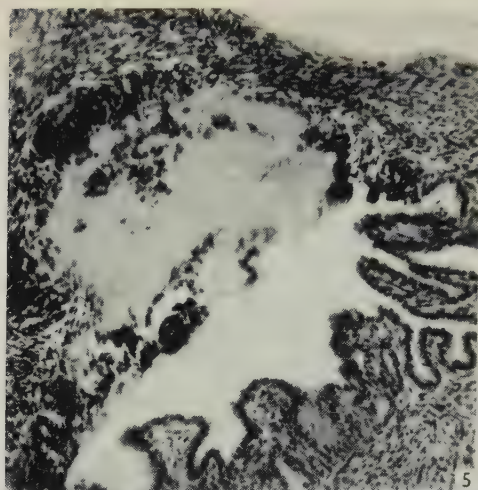
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CLEGG—HISTOLOGY OF SEMINAL VESICLE AND COAGULATING GLAND IN RAT

(Facing p. 366)



CLEGG—HISTOLOGY OF SEMINAL VESICLE AND COAGULATING GLAND IN RAT

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EXPLANATION OF PLATES

All the histological sections from which photomicrographs were made were stained with Ehrlich's haematoxylin and eosin.

PLATE 1

- Fig. 1. Section through the urogenital sinus region of a 5-day-old rat. Dorsal to the sinus (*S*) may be seen the vas deferens (*D*) and seminal vesicle (*V*). The duct of the coagulating gland (*CG*), which possesses a lumen on both sides, may be seen immediately dorsolateral to the urogenital sinus. $\times 60$.
- Fig. 2. Section through the seminal vesicle and coagulating gland of a 10-day-old rat. Although the vesicle possesses a lumen, the acini of the coagulating gland are uncanalized. $\times 150$.
- Fig. 3. Section through the wall of the seminal vesicle of a 21-day-old rat. Fluid separates the epithelium from the submucosa. The epithelium is deficient over a considerable area, and in regions where it is present many nuclei stain more densely than normally. $\times 300$.
- Fig. 4. Section through an acinus of the coagulating gland of a 21-day-old rat. Although in most of the cells the cytoplasm is lightly stained and granular, and the nucleus vesicular, with a prominent nucleolus, three cells possess densely basophilic nuclei with no visible detail, and homogeneous deeply eosinophilic cytoplasm. $\times 1000$.

PLATE 2

- Fig. 5. Section through the seminal vesicle of a 30-day-old rat. Over a considerable region the epithelium is destroyed, and much cellular debris is present in the lumen. Structureless eosinophilic material may be seen adhering to the damaged area and partly filling the lumen of the organ. $\times 150$.
- Fig. 6. Section through the coagulating gland of a 29-day-old rat. Some of the acini appear normal, but others show complete or partial disorganization of the epithelium. $\times 180$.
- Fig. 7. Section through the mucous membrane of the seminal vesicle of a 37-day-old rat. Fluid can be seen deep to the epithelium and separating it from the submucosa. $\times 600$.
- Fig. 8. Section through the wall of the seminal vesicle of a 43-day-old-rat. The membrana propria is covered by a layer of cells which are smaller than usual, whose nuclei are rounded, but which show no evidence of degenerative changes. $\times 430$.
- Fig. 9. Section through the coagulating gland of a 41-day-old rat. A reduplication of the epithelium has become detached from the membrana propria and is separated from it by a structureless material. $\times 425$.

THE HEPATIC VEINS IN MAN AND THEIR SPHINCTER MECHANISMS

BY JAMES B. GIBSON

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The structure of the hepatic veins differs from that of most other veins and resembles that of the hepatic portion of the vena cava, for the muscle is relatively well developed and lies chiefly in the adventitia. This arrangement of the muscle may permit the veins to play a part in controlling the hepatic circulation. Folds are present where the ostia join the cava and a throttle mechanism has been postulated at this level. The older views on this ostial sphincter have been reviewed by Franklin (1937). At a lower level in the hepatic-venous tree, Deysach (1941) described in animals a sluice mechanism which altered the venous drainage under certain experimental stimuli. Those who have studied the hepatic circulation in animal livers by transillumination (e.g. Knisely, Bloch & Warner, 1948; Seneviratne, 1949-50) have referred to a contractile power on the part of the sinusoids especially at their terminations in the central venules (outlet sphincters).

This paper deals with the anatomical basis for sphincter action in the hepatic-venous system in man and its possible function. It reports personal observations on the gross and microscopical structure of the veins and their radicles. Useful accounts of the microscopical structure of the hepatic veins are given by Pfuhl (1922), by Miyake (1928-30) and by Tischendorf (1939), but the subject is confused by a diversity of terminology and by some differences in the reported facts. A revised terminology is proposed to conform to the observed facts. The general arrangement of the large veins and their ostia is well known and has been described by Elias & Petty (1952) and by Gans (1955), and the drainage territories of the main hepatic veins have been defined by Knopp (1953).

MATERIAL AND METHODS

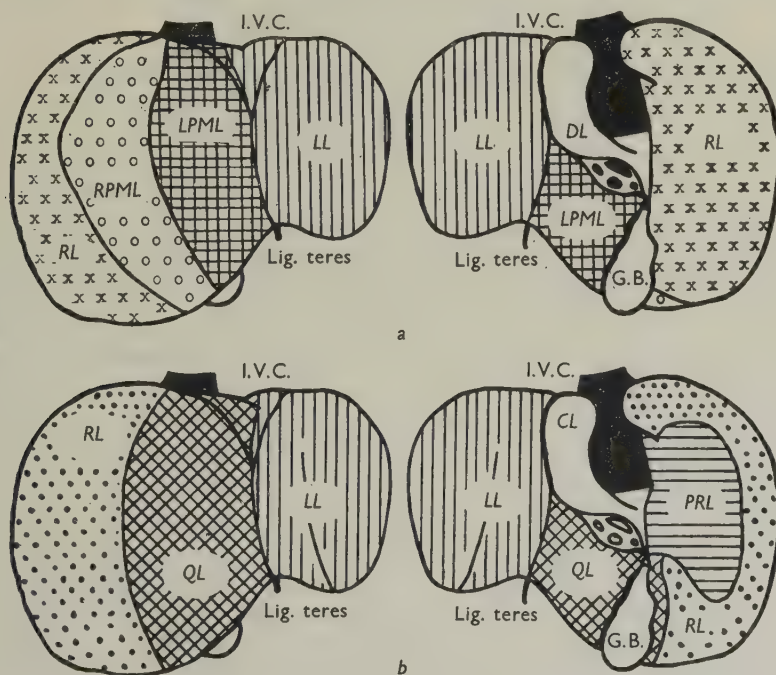
The gross characters of the hepatic veins were studied in approximately 1000 routine autopsies. In addition, in fourteen normal livers obtained at post mortems, retrograde injections were made into the main hepatic veins with plaster of Paris (1), plastic (1), neoprene latex (5) and warm coloured gelatine (7). Injection pressures of 5-22 mm. Hg were used and the livers were washed out through the cannulae for about 15 min. before injection; longer washing produced swelling of the parenchyma. Except in the case of the plaster-of-Paris cast, the relative positions of the veins were preserved by supporting the livers in warm water or saline during injection. The livers were supported and fixed overnight in cold formalin which was acidified for the latex casts. The livers injected with gelatine were sectioned by hand in horizontal planes and the courses and territories of the injected vessels were plotted in relation to the outlines of the livers. For the other preparations liver tissue was removed by dissection and by treatment with acid to leave vascular casts

which were examined under various magnifications. Camera lucida drawings were made of appropriate portions. About 100 blocks selected from normal livers in the autopsy material were studied histologically by a variety of staining methods for elements such as muscle (picro-Mallory method of Lendrum & McFarlane, 1940), elastica, reticulin and nerve. The measurements of the diameters of the veins given in this paper are for the dilated state unless otherwise specified, and were made either directly on casts or on the images of casts projected on the camera lucida. Measurements were made also on paraffin sections and these have been increased by 20 % to allow for shrinkage, a proportion arrived at by a comparison of corresponding vessels in casts and sections.

OBSERVATIONS

Ostia and large hepatic veins

The territories of drainage of these vessels are shown in Text-fig. 1*b*. The right ostium measures about 15 mm. in diameter and the left ostium about 13 mm. The only noteworthy variations in the ostia lie in the extent to which the terminations of the v. hepatica sinistra (*HS*) and the v. hepatica media (*HM*) combine to form the left ostium. Completely separate openings of the veins in the cava were not



Text-fig. 1*a*. The lobation of the liver based on portal-venous supply, according to Gans (1955), but simplified. *RL*, right lobe; *RPML*, right paramedian lobe; *LL*, left lobe; *LPML*, left paramedian lobe; *DL*, dorsal lobes.

Text-fig. 1*b*. The lobation of the liver based on hepatic-venous drainage as worked out in this study. *RL*, right lobe (vein *HD*); *CL*, caudate lobe and process; *QL*, quadrate lobe (vein *HM*); *PRL*, paracaval portion of right lobe; *LL*, left lobe (vein *HS*).

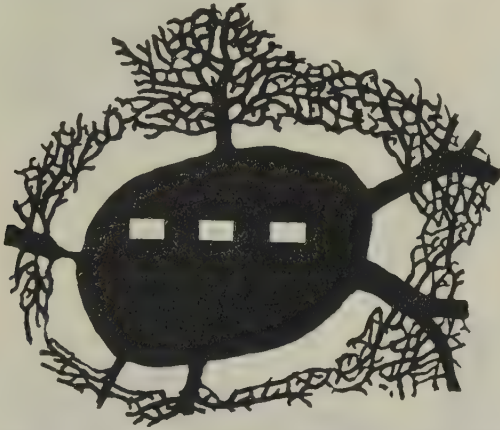
encountered. There is considerable variation in the exact courses and tributaries of the three main hepatic veins, but each of them (*HD*, *HS* and *HM*) regularly drains a certain portion of the liver. The exact boundaries of these portions vary by 1 or 2 cm. from one liver to another. The areas are not the same as those defined by the portal-venous supply. The hepatic-venous radicles alternate with the portal-venous branches at all levels in the liver and with few exceptions, such as the left lobe, the respective territories overlap (Text-figs. 1*a*, *b*). It is noteworthy that the paracaval portion of the right lobe (*PRL* in Text-fig. 1*b*) and the caudate lobe and process are drained by several small ostia located in the cava distal to the two main ostia.

The thickest part of the walls of the ostia and hepatic veins is the muscular adventitia. There is no discrete external elastic lamina or medial coat, but internal to the adventitia there is a zone of moderate thickness which contains fine, circularly arranged smooth-muscle fibres intermingled with elastic and collagenous fibres. In the ostia the elastic fibres of the internal part of this zone are often condensed to form an internal elastic lamina between the circular fibromuscular layer and the endothelial lining, but the lamina is not regularly visible in the hepatic veins. The adventitial muscle is disposed in longitudinal bundles which cause a ridging of the lining of the veins that may be visible to the naked eye. The bundles are bound together by the collagenous and elastic tissue of the internal collagenous layer of the adventitia. External to the longitudinal muscle layer lies a zone of loose texture containing lymphatics, arteries and nerves. The nerve bundles are small and contain fine non-myelinated fibres of autonomic post-ganglionic type. Nerves are most numerous round the ostia but can be found readily in the walls of veins as small as 5 mm. Lymphatics can sometimes be identified in the walls of veins as small as 2 mm. diameter. The outermost layer of the adventitia is composed of collagen and elastic fibres densely adherent to the liver and is in fact a part of the liver capsule. Small bile ducts can sometimes be found in this layer in otherwise normal livers.

Because of the loose texture of the tissue between the internal and external collagenous layers of the adventitia, the inner parts of the ostia and the main hepatic veins are not firmly fixed to the liver substance. This can be appreciated better in fresh than in fixed material. The terminal part of the left hepatic vein lies on the superior surface of the liver and is free to contract, but there is also a considerable degree of play round the lips of both the main ostia. At these lips the muscle coat projects inwards (Pl. 1, figs. 1, 2*a*, *b*) in folds that are better formed at the lower edge of the opening than at the upper. The folds contain two separate layers of muscle, one contributed by the caval and the other by the ostial wall: a few of the muscle fibres are looped round the ostial opening to form a sling (Pl. 1, fig. 2*b*). Between the layers the zone of loose texture is particularly wide, and fat may be present in it. These arrangements constitute the ostial sphincters. Asymmetrical bundles of muscle as in the adrenal and renal throttle veins are not present. The muscle layer of the main ostia is about 0.3 mm. thick, i.e. about half the thickness of that of the caval wall. There is an equivalent abrupt reduction in muscularity where the main radicles join the ostia distally, and folds similar to those at the proximal ends of the ostia are present but they are smaller and less mobile.

The medium-sized and small hepatic veins

The structure of the medium-sized and small hepatic veins is similar to that of the larger vessels, but is progressively simplified in the diminishing orders and the veins are more closely adherent to the liver substance. There are no valves in the hepatic-venous system. Hepatic veins contain more muscle than do intra-hepatic portal branches of similar size. The muscle is progressively reduced, and in veins of of less than .1 mm. diameter the adventitia is composed chiefly of collagenous tissue (Pl. 2, figs. 5, 6). In picro-Mallory stained sections, longitudinal adventitial muscle fibres are rarely found in veins smaller than 1 mm., but circular fibres are often visible in the inner layer at that level and can be distinguished in the smallest of the hepatic veins (400μ). The hepatic veins increase gradually in calibre and join each other and larger trunks in many different ways. The angles of junction tend to



Text-fig. 2. Camera-lucida drawing of part of a thick section of a neoprene-latex cast from an adult human liver. Central venules of various shapes and sizes join an hepatic vein of the seventh order at approximately right angles. Sinusoids do not enter the hepatic vein directly. The central venule on the left is constricted where it enters the larger vessel. Each division of scale (centre) represents 100μ .

become less acute as the vessels are traced down; they approach 90° in the case of the smallest hepatic veins. A feature of the ramification that can readily be appreciated in casts is the fact that many small tributaries—intercalated veins and central venules—join the hepatic veins directly. These junctions are frequent in the case of the smaller orders of hepatic veins, but are also found in medium-sized vessels (Pl. 1, fig. 3). The small vessels enter the larger ones at right angles in a radial fashion (Text-fig. 2). Some are narrowed at their junctions, apparently because their thin walls have been compressed there by contraction of the muscle in the walls of the hepatic veins. These narrowings, termed 'junctional constrictions' in this paper, affect the central venules more than the intercalated veins. They can be seen readily in paraffin sections (Pl. 2, figs. 4–6).

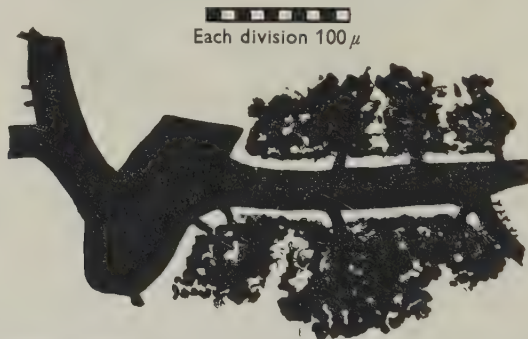
The small veins

For reasons discussed below, the term 'intercalated vein' is applied here to all veins of $100\text{--}350\mu$ diameter. By ordinary histological methods muscle cannot be

demonstrated in the intercalated veins. The vein wall is collagenous and may appear asymmetrical in cross-section; it contains small numbers of elastic fibres arranged circularly. Central venules (40–100 μ) have thin collagenous walls, and elastic fibres are rarely found in them in normal livers. Muscle fibres cannot be demonstrated histologically at any point in the central venules and in particular not at the entries of sinusoids where Knisely *et al.* (1948) located their outlet sphincters in animals.



Text-fig. 3. Camera-lucida drawing of a 'massive lobule' of Pfuhl (1922) and its central venule joining a long intercalated vein. Sinusoids do not enter the latter. From a peripheral area of a neoprene cast of the hepatic veins of a 3-day-old infant.



Text-fig. 4. Camera-lucida drawing of central venules draining lobules of various sizes directly into an intercalated vein which does not receive any sinusoids. Two central venules join an hepatic vein of the seventh order which is the largest vessel shown. From a central area of the same cast as Text-fig. 3.

In some portions of the neoprene casts the injection mass flowed into the ends of the sinusoids. The sinusoids, which were identified microscopically in tease preparations and in thick frozen sections of the casts, terminated only in central venules (Text-figs. 2, 5*a, b*). The sinusoids in the casts formed a continuous sponge-work which was separated from the intercalated and hepatic veins by a space about 100 μ wide. The veins were entirely free of sinusoidal insertions although joined to the anastomosing mass of sinusoids at frequent intervals by hair-like central venules (Text-figs. 2, 4). Examination of paraffin sections confirms that sinusoids terminate exclusively in central venules. There is no channel which can be interpreted as a

vessel intermediate between sinusoids and central venules, but the composition of the latter is diverse and irregular and the largest venules are made up by the junction of smaller ones (Text-fig. 3). These united venules continue to receive sinusoids till close to their terminations in hepatic or in intercalated veins. Many intercalated veins throughout the liver drain into other and larger intercalated veins (Text-figs. 5*a*, *b*) and not directly into hepatic veins, and such large intercalated veins probably correspond to the 'collecting veins' of other authors. They are not, however, characterized by any difference in structure from the smaller intercalated veins which might justify the use of a separate name.



Text-fig. 5. Tease preparations of portions of a neoprene cast of the hepatic veins of an adult. In some instances the sinusoids have been traced in round the central venules. In (*a*) from a central area, an hepatic vein of the eighth order is made up chiefly of intercalated veins of different sizes. Central venules join both types of vein. In (*b*) from a peripheral portion of the same cast, two intercalated veins unite to form an hepatic vein of the eighth order, which in turn joins a larger vein. The intercalated veins are made up by smaller intercalated veins and by central venules. The intercalated veins tend to be longer in this part of the cast and the junctions are more acute.

In the casts, certain small differences between the superficial and the central portions of the liver were noted in the arrangement of the small veins (Text-figs. 5*a*, *b*). In the central parts, a large proportion of the central venules, probably the majority, terminated in hepatic veins, whereas in superficial areas a higher proportion ended in intercalated veins. In the superficial zones, the central venules were more irregular in size and the intercalated veins were often longer and their junctions more acute (Text-figs. 5*a*, *b*). From the above descriptions it is clear that blood leaving a central venule often reaches an hepatic vein, and sometimes an hepatic vein of large size, without having traversed an intercalated vein. In other instances, especially in the periphery, it passes through an intercalated vein before reaching an hepatic vein.

Anastomoses between different venous territories

When warm gelatine of a different colour was injected into each of the major ostia, the mass often entered the sinusoids. The colours sometimes mingled at the limits of the venous territories shown in Text-fig. 1*b* over as much as 2–3 cm. This spread

apparently took place through the sinusoids which form a continuous anastomotic network throughout the liver, and not through larger vessels. Spread was limited because of the small size of the vessels involved but was usually sufficient to fill the sinusoids in the paracaval portion of the right lobe (*PRL* in Text-fig. 1*b*); the proper veins of this area were usually not injected. Evidence was not found of any regular anastomoses between central venules, intercalated veins or hepatic veins within the liver. After full maceration the neoprene casts separated easily into discrete shrublike masses comprising the smaller tributaries of larger veins (Pl. 2, fig. 7). In the occasional instances in which anastomoses were found deep in the liver between small hepatic or intercalated veins, they were associated with other minor irregularities and so are thought to be due to pathological processes. In the earlier stages of cleaning the casts, however, and on the surfaces of livers injected with coloured gelatine, scanty and irregular but definite networks of veins of the size of small hepatic or intercalated veins were usually visible. This capsular network was probably accentuated by the pathological thickening of the capsule which is common particularly on the anterior surface of the liver, but it appeared to be a normal structure and it was found in the single instance of a neonatal liver used in the series. No material difference in respect of the features described in this paper was noted between this cast and the others made from adult livers.

DISCUSSION

Terminology

The *hepatic veins* comprise the section of the venous system between the intercalated veins and the ostia and their structure is uniform in plan. The veins increase in calibre gradually by the junction of radicles, but are conventionally divided into orders. If we suppose that each order measures 1.6 times the diameter of the one below, we find that there are eight orders from the smallest ($400\ \mu$) to the largest hepatic veins (1 cm.). There is a sharp transition at the $400\ \mu$ level from the hepatic veins which are partially muscular to the small veins below them which have walls without muscle fibres. These small veins are of two sorts, intercalated veins and central venules. No important alteration in structure or arrangement was noted in the present study at any level between the central venules and the hepatic veins, and all veins at this level have been described as *intercalated veins* in this paper and the term 'collecting vein' has not been used. The intercalated veins are characterized by the absence from their walls of muscle demonstrable by histological techniques and by the absence of sinusoidal tributaries. The alternative term, 'sublobular vein', seems less apt and has been used by Deysach (1941) to describe vessels in his experimental animals which are clearly hepatic veins. Deysach (1941) reported what appeared to be new channels of drainage for the sinusoids, simple endothelial tubes which he called 'small sluice channels' emptying into sublobular veins. These channels were widely opened by adrenalin and closed by parasympathomimetic drugs. By measurement of the vessels he illustrated, however, it can be seen that the 'small sluice channels' are central venules which can be contracted at their points of entry into hepatic veins (Deysach's muscular sublobulars) in the same way as the junctional constrictions described in this paper. Deysach's 'large sluice channels' were intercalated veins.

The *central venule* is clearly a distinct type of vessel and the present study confirms the findings of Elias & Popper (1955) that they are the only vessels in which sinusoids terminate in man. They are not, however, uniform in size and vary from 40 to 100 μ , the largest venules being made up of smaller ones. Pfuhl (1922) also noted this irregularity in the outflow tracts of the 3-dimensional sinusoidal network. It tends to compensate for inequalities in the venous drainage of a lobular system deriving its blood supply from portal tracts. In parallel with this variation in the central venules, the terminations of the sinusoids are also irregular, for sinusoids enter the venules sometimes singly and sometimes in groups.

Structural basis for the distribution of some hepatic lesions

In cases of hepatic-venous occlusions, areas of liver tissue which correspond to the territories shown in Text-fig. 1*b* are found to be congested or atrophied, while the remainder of the liver is spared or has undergone hyperplasia. For practical purposes the lobes shown in Text-fig. 1*b* are independent in their venous drainage. In the present study, anastomoses other than sinusoidal and subcapsular anastomoses were found only rarely and irregularly between individual hepatic veins (Pl. 2, fig. 7), and this is in keeping with the findings of Tori (1955) and of Goldsmith & Woodburne (1957), though at variance with those of Mall (1906), Elias & Petty (1952) and Gans (1955) who reported frequent anastomoses between small hepatic veins. Structural differences between the superficial and central portions of the liver can often be seen in morbid processes (Walker, 1958) and they may be due, at least in part, to the differences in venous drainage between these areas (Text-figs. 5*a, b*) and to the existence of venous anastomoses in the liver capsule. A difference in reaction between the superficial and deep parts of the liver cannot be ascribed in man to the operation of the vascular-shunt system described by Daniel & Pritchard (1951) in animals. This short-circuit route for blood flow through the liver depends on the close spatial relationship of large portal to large hepatic veins throughout their courses and on their inter-communication through small branches and radicles located at the proximal ends of the large vessels. Although small radicles join hepatic veins of various orders in man (Pl. 1, fig. 3), the courses of the large portal and hepatic veins are not parallel in the unitary human liver as they are in the multilobed livers of animals. The two sets of vessels run at approximately right angles (Elias & Petty, 1952) and the ostia are widely separated from the porta so that there is no common vascular hilum in man.

Pathological patterns on a finer scale can also be explained on an anatomical basis. Elias & Popper (1955) have shown how the regular pattern of chronic venous congestion in man depends on the fact that sinusoids terminate exclusively in central venules in the human liver. In cardiac cirrhosis a significant amount of the new fibrous tissue is formed in the parenchyma in the immediate neighbourhood of the hepatic veins (Moschcowitz, 1952) and this seems to be due to the fact that many central venules join hepatic veins directly (Pl. 1, fig. 3). The lobules served by these venules are exposed more directly than other lobules to the elevated pressure in the larger veins because the junctional constrictions of their central venules are held open in the walls of the dilated hepatic veins.

Hepatic-venous sphincters

Experiments on animals, which have been reviewed by Andrews, Hecker, Macgraith & Ritchie (1955, 1956) strongly suggest that hepatic-venous barriers exist in several species and that they can act under physiological conditions, at least as a variable element of a general response by the hepatic vasculature. Blood flow in such experiments has generally not been facilitated beyond what normally prevails, and the hepatic-venous sphincters would appear usually to be in a state of tonic rather than of active contraction. The vasomotor action is probably under nervous control (Banfai, Kubik & Somogyi, 1953). Hepatic-venous spasm may be important under pathological conditions as in Chiari's disease and in some anaphylactic reactions, but there is no direct evidence for a physiological action on the part of the hepatic veins in man. There are three levels at which the hepatic-venous tree might control the outflow of blood: (i) at the ostia, (ii) at the level of sinusoids, and (iii) at junctional constrictions in the walls of hepatic veins.

(i) Venous spasm has been reported at the ostia (Bradley, Inglefinger, Bradley & Curry, 1945) during hepatic-venous catheterization and has been produced post-mortem by Elias & Feller (1931), but the amounts of muscle present in this site (Pl. 1, fig. 2*a, b*) seem smaller than would be expected if their action played a part in the daily economy of the body.

(ii) Adjustments of the venous outflow at multiple points in small vessels would seem more effective than control at a few sites in the larger veins, but there are no contractile elements in the sinusoidal walls that are demonstrable by histological methods. The anatomical basis for the outlet sphincters described by Knisely *et al.* (1948), Wakim & Mann (1942) and Bloch (1955) in transillumination studies on animals remains obscure. The sinusoidal circulation of the liver, however, operates at pressures under 40 mm. Hg so that the critical opening pressure is probably above the critical closing pressure (Burton, 1954). This factor will tend to maintain any closure of the sinusoids that follows a reduction in the entry of blood, and the latter is probably the primary factor causing narrowing of the sinusoids (Andrews, 1957). The possibility cannot be excluded that changes in the shape or alignment of the liver cells themselves can occur under physiological conditions in such a way as to alter the diameter of the sinusoids to which they are so closely applied.

(iii) The experiments of Deysach (1941), of Macgraith, Andrews & Wenyon (1949) and of Thomas & Essex (1949) have shown that the venous drainage can be altered round individual hepatic veins in animals. In man, Popper (1931) described junctions of central venules with hepatic veins as 'funnel-like' and later with Elias (1955) illustrated thin-walled venules constricted on entering a thick-walled vessel. In the neoprene casts made in the present study central venules were found commonly to enter hepatic veins (Text-fig. 2; Pl. 1, fig. 3) and these junctions were sometimes contracted (Text-fig. 2). Both dilated and contracted junctions were seen in sections (Pl. 2, figs. 4-6). The junctions are so frequent that junctional constriction is probably the chief venous-sphincter mechanism in the human liver. The free sinusoidal anastomosis will tend to equalize the effects among lobules in the immediate vicinity of any single constriction. Constrictions are absent where thin-walled central venules or intercalated veins unite, and thus the arrangement of the

finer radicles may influence the effectiveness of the sphincter mechanism in different parts of the liver. Circulation through the peripheral portions can be affected less by junctional constrictions than that in the central portions, because towards the surface a greater proportion of the central venules joins intercalated veins (Text-fig. 5a, b).

Possible physiological action of the hepatic veins

The special distribution of the muscle of the hepatic veins of man in longitudinal bundles seems to suit the vessels for a more or less automatic action concerning respiration. There are no valves in the hepatic veins and they are exposed to respiratory variations in thoracic pressure. Brecher, Mixter & Share (1952) have pointed out the importance of collapse of extra-thoracic veins such as the axillary and jugular veins, but this is not possible in the case of the hepatic veins, which are firmly attached to the liver substance. When blood is sucked out of the hepatic veins in inspiration, contraction doubtless occurs in the vessel walls to preserve tone. In this way, the central venules are narrowed at their junctional constrictions and the negative pressure is damped down before affecting the sinusoids. At rest, hepatic-venous constriction is probably not essential, but on deep breathing the barrier action of the hepatic-venous muscle may play a valuable part in protecting the liver from the greatest fluctuations in caval venous pressure and, to a lesser extent, in preserving the autonomy of the portal circulation.

SUMMARY

The hepatic veins and their sphincter mechanisms are described as seen in naked-eye and microscopical studies on autopsy material supplemented by casts of the veins and their small radicles and by gelatine-injection preparations.

The only normal anastomoses between the hepatic-venous territories are the continuous sinusoidal network and an irregular network of small veins in the external capsule of the liver. The hepatic veins maintain a uniform pattern of structure throughout and muscle is present chiefly in longitudinal bands in the adventitia. The smallest hepatic veins measure 400μ in diameter in the dilated state.

The smaller veins are of two kinds—the larger or intercalated veins, $100\text{--}350\mu$ in diameter and the central venules, $40\text{--}100\mu$ in diameter. Muscle cannot be demonstrated in their walls by ordinary histological techniques. Central venules are the only veins which receive sinusoids in the human liver. They vary considerably in form and this permits an even drainage of the sinusoidal network deriving its blood from the portal tracts.

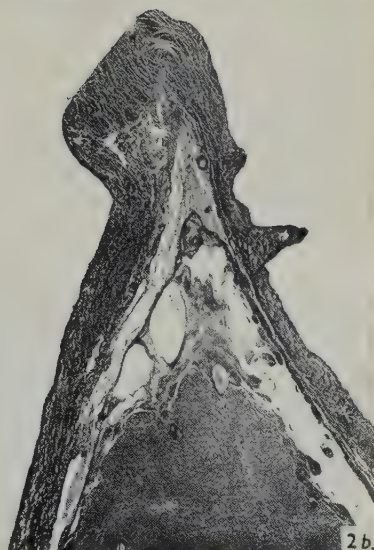
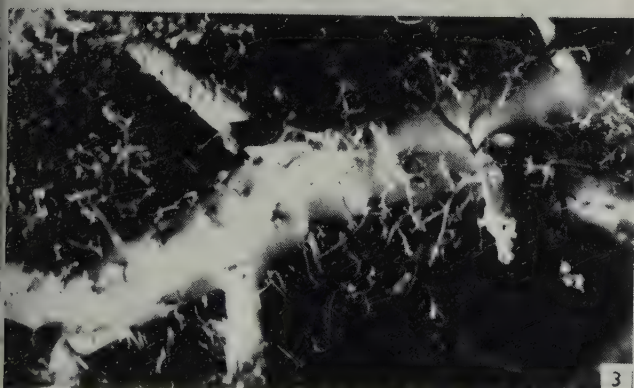
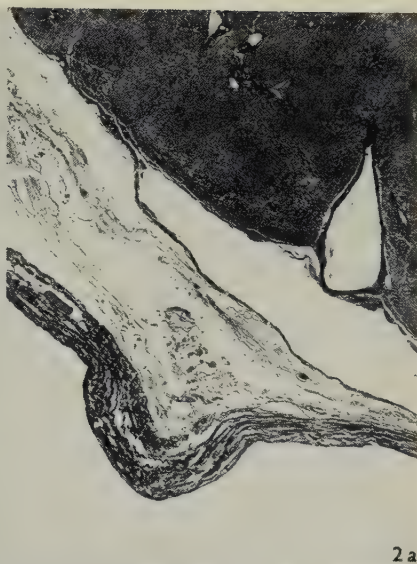
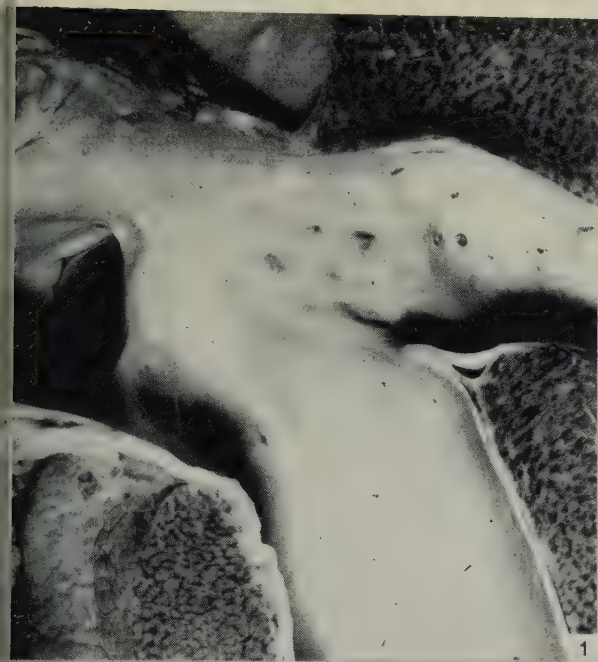
Some structural peculiarities which may determine morphological features of pathological processes in the liver are discussed. On anatomical grounds, it is considered that the short-circuit routes of Daniel & Pritchard (1951) cannot be developed sufficiently in the human liver to be of practical importance.

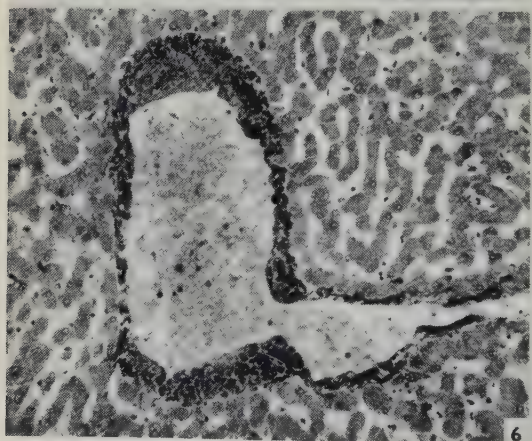
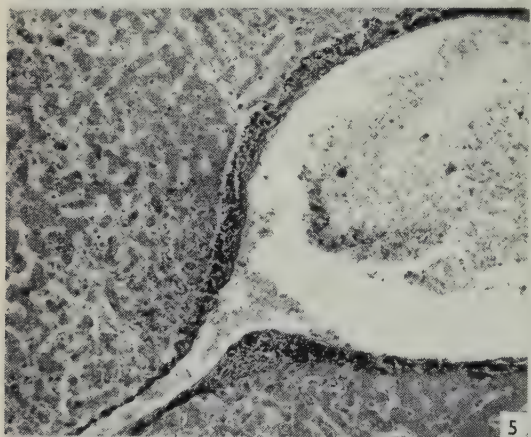
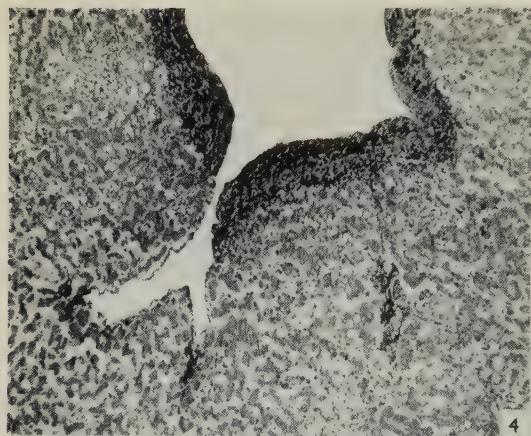
Structures which might act as venous sphincters are present at the ostia and at innumerable points of junction of central venules with small and medium-sized hepatic veins (junctional constrictions). An anatomical basis was not observed for outlet sphincters at the terminations of sinusoids in central venules. The evidence for physiological sphincters at the ostia is unsatisfactory, but junctional

constrictions can probably act in this way. Their action may protect the sinusoidal portion of the vascular bed from the fluctuations in the caval blood pressure caused by respiration.

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GIBSON—THE HEPATIC VEINS IN MAN AND THEIR SPHINCTER MECHANISMS

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Junction of the right hepatic ostium (right) with the hepatic cava, dilated and seen from behind. Parts of the left ostium and of *HM* are visible (left). The distal lip of the right ostium projects as a fold. $\times 1.5$.
- Fig. 2. Longitudinal section of the right hepatic ostium showing folds formed by the junction of the ostial wall (right) with the caval wall. Picro-Mallory stain, $\times 9$. At the upper lip (*a*) the external collagenous layer of the vessel, normally firmly adherent to the liver tissue, has been separated by artefact. The fold at the lower lip (*b*) is larger and contains lymphatics in the loose zone of the vessel wall.
- Fig. 3. A portion of a neoprene-latex cast of hepatic veins of the left lobe. The largest vein (2 mm. in diameter) is an hepatic vein of the fifth order. It receives hepatic veins of smaller size and many intercalated veins and central venules. The central venules are the smallest vessels visible. $\times 4.5$.

PLATE 2

- Fig. 4. A contracted hepatic vein of the sixth order (approximately 0.5 mm. in diameter in this state) which is joined by a dilated central venule of about 60μ in diameter. The latter is constricted at its junction. Picro-Mallory stain, $\times 65$.
- Fig. 5. Dilated seventh-order vein (800μ) and a central venule (50μ). The junction is widely patent. There is slight centrilobular congestion. Picro-Mallory, $\times 80$.
- Fig. 6. Contracted eighth-order hepatic vein (300μ diameter in this state). It is joined by a large central venule which is constricted at its mouth. This is a funnel-like *junctional constriction*. The muscle which is irregularly distributed in the wall of the hepatic vein is restricted to the intima. Picro-Mallory, $\times 110$.
- Fig. 7. Anterior surface of the left lobe of the liver. The hepatic veins have been injected with neoprene-latex and the parenchyma digested down to a depth of about 3 cm. The field is composed of discrete clumps each centred round an hepatic vein of about the sixth order (2 mm.). The radicles form a medusa-head pattern and do not anastomose. The finest vessels clearly visible are central venules. $\times 2$.

LYMPHATICO-VENOUS COMMUNICATIONS IN THE ALBINO RAT

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There has long been disagreement as to whether lymphatico-venous communications—exclusive of the subclavian connexions—are a normal occurrence. Bartels (1909) definitely denies their existence and disputes the claims of earlier investigators who had recorded such anastomoses. Baum (1911, 1912) considered that he had found lymphatico-venous communications several times in the thorax and abdomen of cattle, but he admits that it is often difficult to prove that the dye has been injected into a lymph vessel and not into a vein. There are some reports which indicate that the situation may vary from species to species or group to group. In New World monkeys Silvester (1912) found that the main opening of the mesenteric and lower limb lymphatics was into the inferior vena cava near the renal vein, but in the Old World monkeys no indications of these communications could be found. In the common wild rat, the frequency and localization of lymphatico-venous communications was investigated by Job (1918). In 48% of the rats injected through, or caudal to, the lumbar nodes lymphatic communication with the inferior vena cava just cranial to these nodes was found and 8% showed renal vein communications. In cats, however, Carlsten & Olin (1952) found that the only route for any appreciable amount of intestinal lymph to reach the blood stream was the thoracic duct.

It is of the utmost importance for the evaluation of a number of physiological investigations concerning the lymphatic system and for an understanding of the spread of cancer by this system to determine the occurrence and frequency of lymphatico-venous anastomoses. The literature contains no reports of such investigations in the common laboratory rat, and Job's findings have not been confirmed. This problem has, therefore, been investigated, and by a method different from that previously employed.

MATERIAL AND METHODS

The material included twenty-two rats of the laboratory's hooded strain, bred as a closed population for many years, and five wild rats (*Rattus norvegicus*).

The lymphatics were visualized by injecting metallic mercury into the lymph vessels or nodes. Their course can then be determined both roentgenologically and by dissection (Engeset, 1959). Job (1918) states that lymphatico-venous anastomoses were found most readily in healthy animals which were active just before sacrificing and which were injected immediately after death. He found more anastomoses in animals killed with illuminating gas than in animals killed with chloroform or ether. 'Pregnant females, within a few days of delivery, always showed two or more venous connexions besides the thoracic duct taps.' These points were borne in mind and every effort was made to afford optimal conditions for the demonstration

of lymphatico-venous anastomoses by using healthy rats, pregnant within a few days of delivery. The animals were killed with carbon monoxide after running in a drum for 1–2 min. They were laparotomized immediately after killing, and mercury was injected into the lumbar nodes.

The injection was made with an automatic syringe which delivered 0.01 ml./min. First 0.04 ml. was injected into the right lumbar node and the mercury usually went to the saccus lymphaticus and the thoracic duct, or it could be milked up into the duct. To force the filling of lymphatico-venous anastomoses in the abdomen, the thoracic duct was then ligated in the middle of the thorax, and 0.08 ml. mercury was then injected in the left lumbar node. The animals were X-rayed and the findings were controlled by dissection under magnification.

RESULTS

Series 1 consisted of the twenty-two rats of the laboratory strain. In all of them the roentgenograms showed mercury in a number of lymph vessels and nodes in the abdomen. The saccus lymphaticus was distended with mercury, and in most of the animals was a considerable amount of retrograde filling in the chylus vessels (Pl. 1, figs. 1 and 2).

In fifteen of the animals neither the roentgenograms nor the dissection afforded any evidence of lymphatico-venous connexions in the abdomen.

In seven of the animals drops of mercury could be identified in the large abdominal veins. In none of these, on dissection, however, were the lymphatics observed to empty into the veins. In three of them it was established that the mercury had entered the venous system through small veins in the wall of the left lumbar node, and in one through veins in the wall of the right lumbar node (Pl. 1, fig. 4). In two of the animals the passage was, respectively, through veins in the wall of the right and the left renal node (Pl. 1, fig. 3). In one animal the site of the communication was not found. In this specimen the mercury was seen as a column in the vena cava between the two lumbar nodes. Ventral to the vein there was an abundant network of small, mercury-filled vessels which were connected with the lumbar nodes. In an attempt to follow one of these vessels towards the dorsal side of the vena cava the vein was ruptured and the dissection was spoiled.

Series 2. Five wild rats were investigated in this series. They were pregnant and, like those in Series 1, they were killed with carbon monoxide and the thoracic duct was ligated.

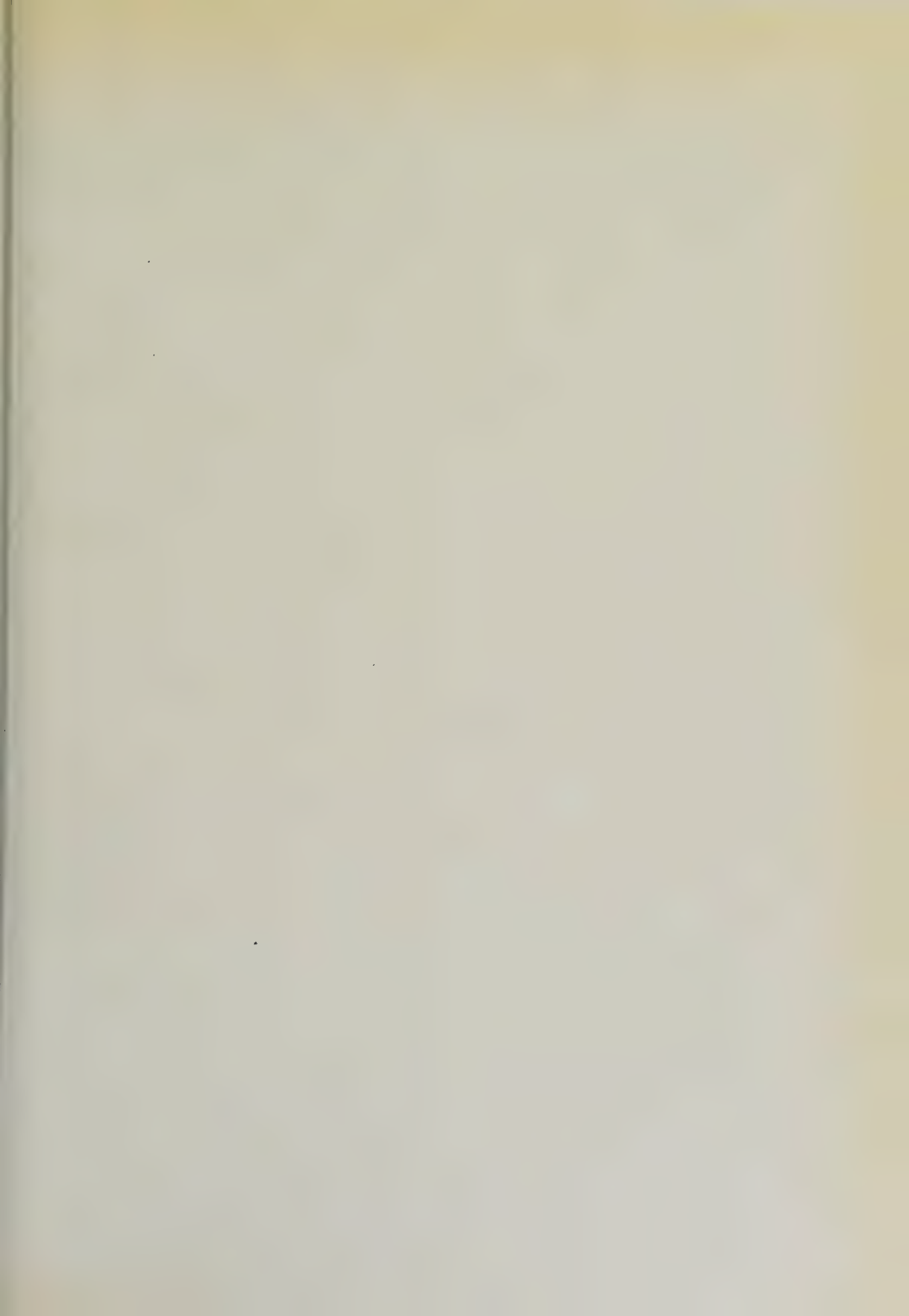
In none of these wild rats were lymphatics observed to empty into abdominal veins. In two animals, however, mercury had entered the vena cava through small veins in the wall of the left lumbar node. In a third rat which showed drops of mercury in abdominal blood vessels, it was not possible to find the connexion between the lymphatic and venous system. In this animal the mercury injection had not been completely successful. Several punctures had been made in the left lumbar node, and it is possible that a small amount of mercury may have been directly deposited in a vein.

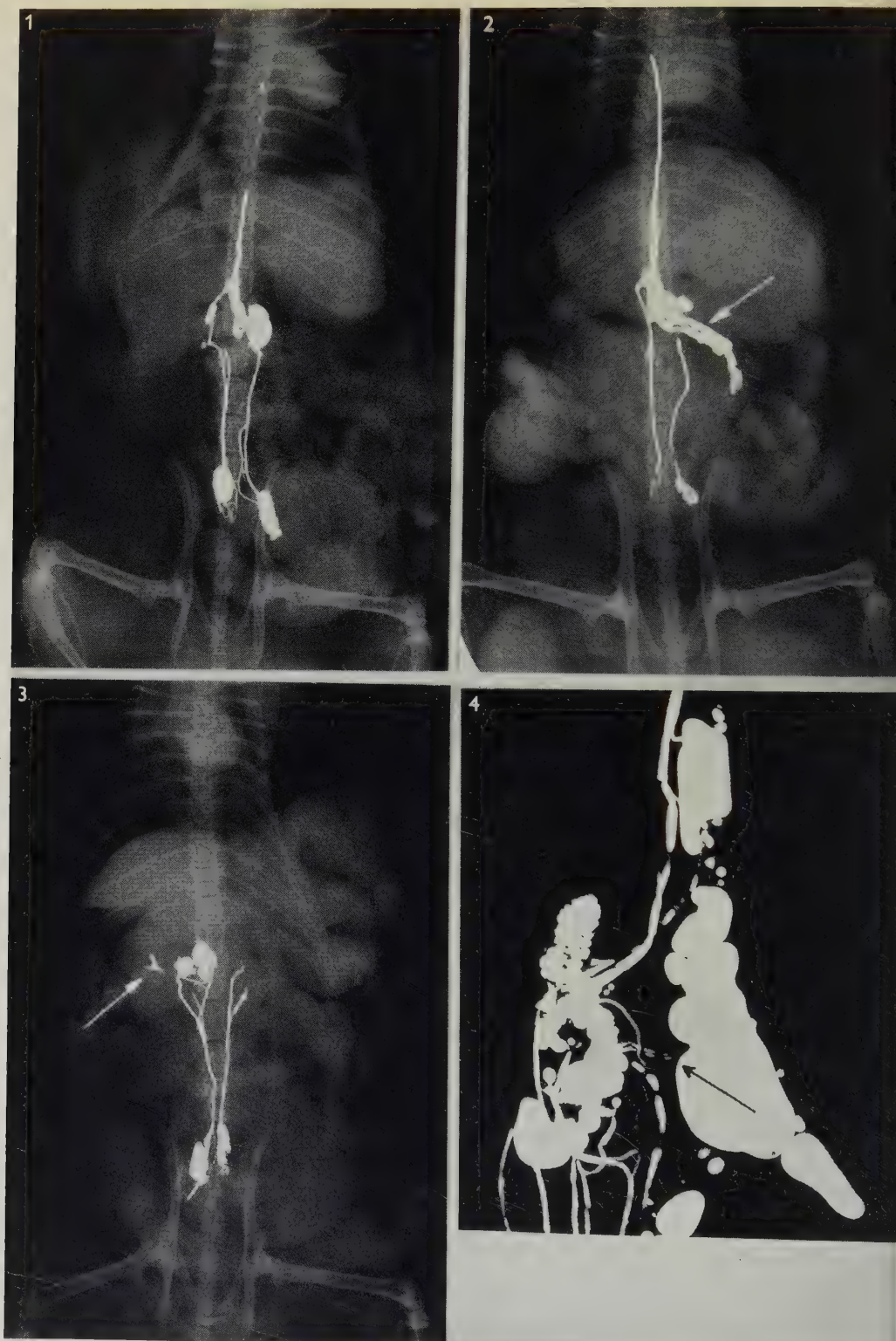
DISCUSSION

Metallic mercury was injected in the lumbar nodes of twenty-two tame and five wild rats to investigate the frequency of lymphatico-venous communications. The topography of the efferent lymphatics was determined roentgenologically and by dissection. In no case was it found that the lymph vessels from the nodes emptied into abdominal veins. This is in contradiction to Job's findings in wild rats: under optimal conditions—which have also been applied in the present investigation—he found lymphatico-venous communications in the abdomen in practically all of the animals he examined. According to Job the lymphatic system of wild rats is much better developed than in captive white specimens. This may explain why the results are so different, but it is surprising that no anastomoses were found in any of the five wild rats examined. Job injected a Berlin blue gelatin mass and indian ink. If the anastomoses to the venous system are of a very fine calibre, this may make a difference, as indian ink would presumably flow more readily through the lymph vessels than mercury. But it is not reported that the lymphatico-venous communications, which have also been demonstrated in other animals, are particularly thin. Moreover, it is known that mercury flows readily in vessels of ordinary calibre.

It is our opinion that mercury affords a more reliable basis for the study of this problem than the injection of various dyes. The mercury does not mix intimately with blood or lymph. For this reason it is relatively easy to determine whether the injection has entered a small blood or lymph vessel. In the cases where the mercury passed into veins in the wall of a node, drops of blood were found between the drops of mercury, and there were side branches which contained pure blood. Further, the passage occurred through veins which ordinarily lead from the node and the topography of which is familiar from numerous injections and dissections (Pl. 1, fig. 4). After injection of mercury the course of the lymphatics can be accurately determined roentgenologically, and even the smallest passage of mercury to the venous system can be visualized. Thus lymphatico-venous communications which allow the passage of mercury will not be overlooked, and it may easily be determined whether a lymph vessel which appears to end in the wall of a vein has emptied into the vein or not. In such cases it is also an advantage that the mercury can be 'milked' forward in the lymph vessels. This has been done several times when the mercury filled a lymph vessel which appeared to end in the wall of the vena cava. By palpation of the end point of the vessel and 'milking' of the mercury, it was possible to follow the further course of the lymph vessel, usually over to the lymphatics on the other side.

Investigation by this method affords such a high degree of accuracy that it is possible to draw the conclusion that lymphatico-venous communications in the abdomen must be very rare in the laboratory strain examined, or they must be of such fine calibre that they do not allow the passage of mercury, even under the optimal conditions provided. In several of the rats the mercury entered the large veins through small veins in the wall of a lymph node. This may possibly be due to the puncture of the node or to lesions resulting from the high pressure used for the injection. Whether it can be due to lymphatico-venous anastomoses in the lymph node itself has not been investigated or considered.





ENGESET—LYMPHATICO-VEIN COMMUNICATIONS IN THE ALBINO RAT

(Facing p. 383)

The observations made can hardly be confined to the strain of rats employed in investigation. After injection of mercury in the knee node and in lymph vessels on the hind-leg of fifty-six rats from another laboratory, not a single case was observed in which lymph vessels emptied into abdominal veins.

SUMMARY

In order to examine the frequency of lymphatico-venous communications in the abdomen, metallic mercury was injected in the lumbar nodes of twenty-two tame and five wild rats. The injections were made under conditions which should afford optimal possibilities for the demonstration of such anastomoses. The topography of the vessels was determined roentgenologically and by dissection. In none of the specimens were the lymphatics from the lumbar nodes observed to empty into abdominal veins. In some of the rats, probably owing to injection artefact, mercury entered the vena cava inferior through small veins in the wall of the lymph nodes.

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EXPLANATION OF PLATE

- Fig. 1. Rat. Mercury injected in lumbar nodes, both sides. Thoracic duct ligated. Saccus lymphaticus distended with mercury. Anastomoses between lymph vessels. No mercury outside the lymphatic system.
Fig. 2. Rat. Mercury injected in lumbar nodes, both sides. Thoracic duct ligated. Note the retrograde filling in the chylus vessels. No mercury outside the lymphatic system.
Fig. 3. Rat. Mercury injected in lumbar nodes, both sides. Thoracic duct ligated. Note mercury outside the lymphatic system in a vein proximally in abdomen.
Fig. 4. Radiogram of the right lumbar node magnified. Most of the node with efferent and afferent lymph vessels filled with mercury. Note that large amounts of mercury have entered vena cava inferior and vena iliaca com. An arrow points to small veins through which the mercury entered the vena cava.

REVIEW

Blastogenese des Menschen. By KAREL MAZANEC. (Pp. ix+179; 118 illustrations; D.M. 20.60.) Jena: Gustav Fischer Verlag. 1959.

Our knowledge of the earlier developmental history of man has been enormously increased in the last twenty-five years. The increase has been due largely to the labours of Hertig and Rock, whose successive publications in the Contributions to Embryology have revolutionized our concepts on human embryology in the two weeks following fertilization. There have, however, been a number of other descriptions of implanted embryos which fill in certain lacunae left in the Hertig-Rock collection. Dr Mazanec summarizes such specimens, including the older classical ones, in the first half of his book and fits them against the background established by the two American investigators. This summary will be of much use to all interested in early human embryology.

The second half of the volume is a discussion of the conclusions that the author has drawn from a study of the described embryos. Not all of these conclusions will be found completely acceptable. Nevertheless, they are an interesting commentary on the valuable list provided in the first half.

W. J. HAMILTON

BOOKS RECEIVED

The Postnatal Development of the Human Cerebral Cortex. Vol. VI. *The cortex of the twenty-four month infant.* 1959. By J. LEROY CONEL. (Pp. 309. £5.) Harvard University Press. London: Oxford University Press.

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Bau und Funktion der normalen Milz. By E. VON HERRATH. 1958. (Pp. viii+412, 124 illustrations. Geb. D.M. 68.) Berlin: Walter de Gruyter & Co.

POSTNATAL DEVELOPMENT OF THE CEREBRAL CORTEX IN THE RAT

By J. T. EAYRS* AND B. GOODHEAD

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Present knowledge of the development and structural organization of the cerebral cortex is based largely on qualitative observations, pioneered by the classical studies of Ramón y Cajal (1911) and later extended by Lorente de Nó (1922, 1949), von Bonin (1948*a*) and many others. Current treatment of the mode of functioning of the central nervous system, however, whether based on cybernetics, analogies with models or on mathematical concepts embracing information theory or conditional probability, lays emphasis on the pattern of connectivity between axons, perikarya and dendrites, and demands a closer knowledge of the quantitative aspects of the inter-relationship between neurones than is provided by purely descriptive neuro-histology.

An early attempt to apply techniques of measurement to finer cortical structure was that of Bok (1936*a, b*), who investigated the relationship between the size and density of perikarya and depth beneath the pial surface, and established the idea of the 'cell territory' expressed in terms of the perikaryon and the extent of its associated dendritic field. These concepts have more recently been applied to the overall pattern of cortical organization (Campbell, 1954; Ryzen & Campbell, 1955) and further developed by Sholl (1953) whose data, combined with those relating to the distribution of axons (Sholl, 1955), have provided an anatomical basis for the estimation of neuronal connectivity (Sholl, 1956). Similar methods have been used in preliminary studies designed to correlate the histological abnormalities arising as a result of experimental cretinism with changes in adaptive behaviour (Eayrs, 1955).

Quantitative techniques have, as might be expected, been applied more freely to the study of changes taking place during development. Some workers have taken as their criteria of measurement the time of appearance of some relatively well-defined landmark of cortical maturation, such as the beginning of myelination (Flechsig, 1920), the first-appearance of Nissl granules (Sugita, 1918*a*; Conel, 1947), a change in the refractive index of dendrites (Peters & Flexner, 1950) and modification of the electrical activity of the brain (Crain, 1952; Flexner, 1955). Others have plotted the course of cerebral development in terms of phenomena which are observable only as continuous processes, such as the increase in the surface area (Smith, 1934; Turner, 1950), volume (Kappers, 1926) and thickness of the cortex and its several layers (Sugita, 1917, 1918*b*; Conel, 1951), in the size of perikarya (Sugita, 1918*a*), in vascularity (Craigie, 1925), and alterations in the cerebral ground substance (Goodhead, 1957).

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An important phase of cortical maturation which has so far received but little quantitative treatment, however, is the growth and ramification of cell processes to form the neuropil and so vastly to increase the probability of synaptic inter-relationship between neurones. The possible functional significance of this aspect of development was first suggested by von Economo (1926) who postulated, on the basis of comparative anatomical studies, that the relationship between the mass of the grey matter to that of the perikarya of its constituent neurones ('cell/grey coefficient') might be used as an index of the organizational status achieved by the brain. Interspecific differences in this coefficient appear to be inversely correlated with the size of the brain (Tower & Elliot, 1952; Shariff, 1953), while within the same species the coefficient decreases with advancing age (e.g. Sugita, 1918*b*; Brody, 1955). Although the prolongation of this phenomenon into old age is probably due to a loss of neurones and their replacement by non-conducting tissue, there can be little doubt that a decrease in cell density during a time when the brain is increasing in size must be associated with an elaboration of neuropil and, from a functional point of view, a corresponding shift of emphasis from the axo-somatic to the axo-dendritic mode of cortical integration whose possible significance has been pointed out and discussed by von Bonin (1948*b*).

These considerations must assume particular importance in any attempt to interpret the changes in cortical structure which result from hypothyroidism arising during early infancy. When this state is induced experimentally in the infant rat the expected decrease in the cell/grey coefficient fails to materialize (Eayrs & Taylor, 1951). Not only is the neuropil as a whole hypoplastic, but the growth of axons appears to be differentially retarded in certain regions of the cortex while, at the same time, the decay in the dendritic field of pyramidal neurones departs from the exponential pattern characteristic of the normal individual (Eayrs, 1955). So little quantitative data are available concerning the pattern of growth of cell processes in the normal individual that it has not been possible to assess whether these anatomical changes represent a distortion or merely a retardation of growth. The studies reported in the present paper have accordingly been undertaken to elucidate the mode of development of the cortical neuropil in the rat from birth to maturity.

MATERIALS AND METHODS

Animals

Forty-five young male rats and five male adults, all of the Birmingham strain, were used for this study. Variation in growth was minimized by breeding all the young from virgin females and raising them in litters reduced, on the day of birth, to a standard size of six.

(a) Histological treatment *Preparation of tissues*

On the day of birth, and at ages of 6, 12, 18, 24 and 30 days post-partum, groups of rats were weighed and killed in chloroform vapour. Their brains were removed, weighed, and subjected to one of the following procedures:

(i) Fixation in a 25 % solution of chloral hydrate in 50 % ethanol and subsequent treatment by the method of Nonidez (1939) for the silver impregnation of

axons. Coronal paraffin sections were cut at 10μ thick, counterstained in Borel's methylene blue and mounted in DPX.

(ii) Processed by the Golgi-Cox method (modification of Sholl (1953)), embedded in celloidin and cut at 200μ in a coronal plane through one hemisphere and in a plane tangential to the frontal cortex through the other.

(iii) Fixation in a solution consisting of 90 parts of 70 % alcohol to 10 parts 40 % formaldehyde, coronal paraffin sections being cut at 10μ thick and stained with gallocyenin.

The brains of the adult rats used were similarly treated.

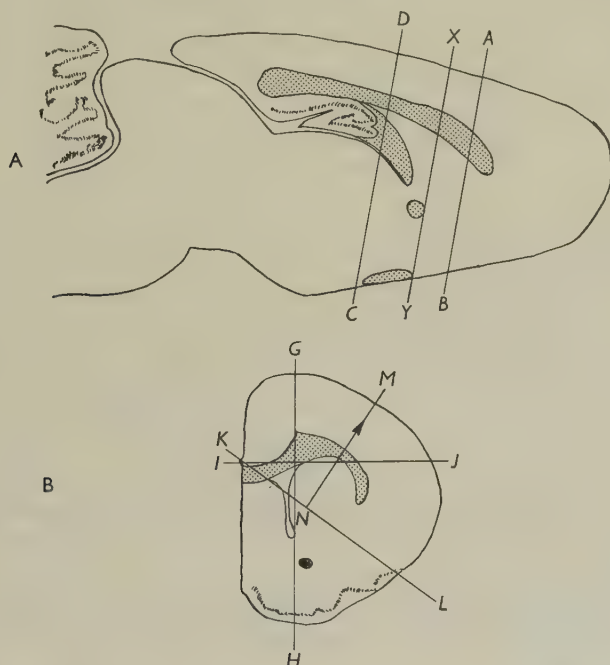


Fig. 1. Semi-schematic diagrams (approx. $\times 5$) showing mode of preparation of tissues for histological examination. The line XY (top figure) marks the plane from which all coronal sections were cut. Sections tangential to the cortical surface were taken from the block of tissue represented by $ABCD$, whose anterior surface is illustrated in the lower figure. Sections were cut from the plane KL along the axis NM (for further explanation see text).

(b) *Planes of section*

Since the density of cortical neuropil varies from region to region care was taken to ensure that comparable areas were studied at all ages. Accordingly, before embedding, each brain was divided sagittally and one hemisphere divided in a plane, orthogonal to its medial surface, passing through the anterior margins of the anterior commissure and optic chiasma (XY in Fig. 1A). Coronal sections were cut from the block of tissue posterior to this plane, and a study made of those in which the anterior limits of the fornix first appeared. This plane of section passes through the sensori-motor cortex, and measurements were made in the region corresponding to that described as area 2 by Krieg (1946*a, b*), which has been previously studied quantitatively by Eayrs (1955) in normal and hypothyroid rats.

The second hemisphere of brains processed by the Golgi-Cox technique was used to obtain sections tangential to the cortical surface. The anterior surface of a block of tissue about 4 mm. thick cut in the coronal plane described above (see Fig. 1A) was divided visually in the manner illustrated by Fig. 1B in which the lines *GH* and *IJ* represent the planes previously used by Eayrs & Taylor (1951) and *M* is the mid-point of the cortical segment lying between them. The line *NM* is orthogonal to the cortical surface at *M* and passes through area 2. The cortex was removed by a cut in the plane *KL* which was subsequently used to orientate the specimen during embedding in celloidin and from which serial sections at 200μ were cut in the direction of *NM* throughout the cortical thickness. Sections passing through layer 5*b* were identified by measuring the depth of this layer beneath the pial surface on coronal sections of the opposite hemisphere and counting off the number of tangential sections corresponding to this depth.

Quantitative estimations

(a) Axon network

The density of the axon network was estimated in a manner similar to that previously described by Eayrs (1955), using an optical system giving a magnification of $\times 900$. With the optical field centred in each of the cortical laminae 1, 3, 4, 5*a*, 5*b*, and 6*a* a grid (100×0.5 mm. squares) set in the focal plane of the eyepiece was used to count the number of axons intersecting a line of given length and to measure the proportion of this length occupied by other readily identifiable elements of the cortical tissue, i.e. perikarya, blood vessels, apical dendrites, and neuroglial nuclei. This proportion was subsequently used as a correction factor (see Table 4) to convert the number of axons counted within the cortex as a whole to that within the neuropil itself. The data were subsequently standardized, by taking into account the depth of focus of the optical system, to express the number of axons cutting an area of cortex of 1 mm.^2 .

Estimates were carried out in different parts of each lamina; recounts later made on a number of randomly selected sections fell within 5% of the original values.

(b) Cell/grey coefficient

The cell/grey coefficient was measured by recording the nature of the tissue underlying the intersections of the lines forming the grid. The number of coincidences between an intersection and a cell body relative to the total number of intersections was taken as an estimate of the proportion of cortical tissue occupied by perikarya.

Tests of reproducibility of the estimates were made by means of sample counts and gave results similar to those for axons.

(c) Dendritic fields

The dendritic fields formed by the basal dendrites of pyramidal cells in layer 5*b* were measured on coronal sections by the method described by Eayrs (1955) to estimate (i) the number of dendrites arising from the perikarya, (ii) the dendritic density, and (iii) the mean occurrence of points of branching and ending at successive intervals of 18μ from the centre of the perikaryon. Similar observations were made on neurones in sections cut tangential to the cortical surface and identified by

measurement and inspection as passing through the middle of layer 5*b*. Ten randomly selected cells, five coronally and five tangentially orientated, were measured in this manner for each rat, the means of the data so derived being taken to characterize the dendritic fields of pyramidal cells in the tissues of the frontal cortex.

RESULTS

(a) *Qualitative changes in cortical structure*

(i) *The cerebral cortex at birth*

At birth local cortical differentiation has barely commenced, and cells are small and closely packed. Of the several laminae, only layer 1 can be distinguished with ease; layers 3 and 4 cannot be separated and consist of cells, strongly chromophilic, which are arranged in orderly columns. The position of a developing layer 5 can be identified as a thin band of somewhat larger neurones, while layer 6 is represented by a zone of cells in which Nissl substance is scanty and whose arrangement lacks any of the orderly pattern of those in layers 3 and 4. Very few axons indeed can be seen in the outermost layers of the cortex, and although considerably more are present in the deeper layers most of these fibres run a tangential course and no radially orientated bundles of axons (specific afferents of Lorente de Nó, 1922) are visible. The corpus callosum is poorly developed with few axons impregnated, its definitive position being largely occupied by migrating neuroblasts. No conclusion could be reached concerning the growth of dendrites, for Golgi-Cox preparations consistently failed to reveal any cells at all.

(ii) *The cerebral cortex at 6 days of age*

By the sixth day a marked increase in cortical differentiation has occurred and most of the laminae characteristic of the adult can be recognized without difficulty, although the perikarya are still closely packed together. The boundary between layers 3 and 4 is marked by the presence of larger cells which are apparently destined to develop into the 'border pyramids' found in this situation in the adult. Layer 4 itself is beginning to present its characteristic granular appearance and some of the cells in layer 5 are assuming a pyramidal shape, although none is yet argyrophilic as is the case in the adult. These cells presumably belong to sublayer 5*b* and no sublayer 5*a* (lamina interstriata), which is so characteristic of this region in the mature rat (Vaz Ferriera, 1951), has yet developed.

The number of axons has increased markedly since birth in all layers of the cortex, but bundles of radially orientated fibres traversing layers 5 and 6 have still not appeared. Most of the neurones demonstrated by the Golgi-Cox technique are pyramids located in layer 5. Each has a short apical dendrite which does not extend as far as layer 1, and does not branch. Few basal dendrites are present, many cells possessing none at all (Fig. 2).

(iii) *The cerebral cortex at 12 days of age*

The appearance of the cortex in the 12-day-old rat is, in general, characterized by: (1) an increase in the size of the perikarya; (2) a reduction in the packing density; and (3) a more precise demarcation between the several laminae, the lamina

interstriata now being well differentiated. The cells of the infragranular layers have developed conspicuous amounts of Nissl substance, and there is a marked tendency towards pyramidization in layer 5*b*, where a few cells now possess the argyrophilic properties of the adult. A marked increase in axon density has occurred, particularly in the granular and infragranular layers where many radially orientated fibres are now present, though not conspicuously assembled into fasciculi.

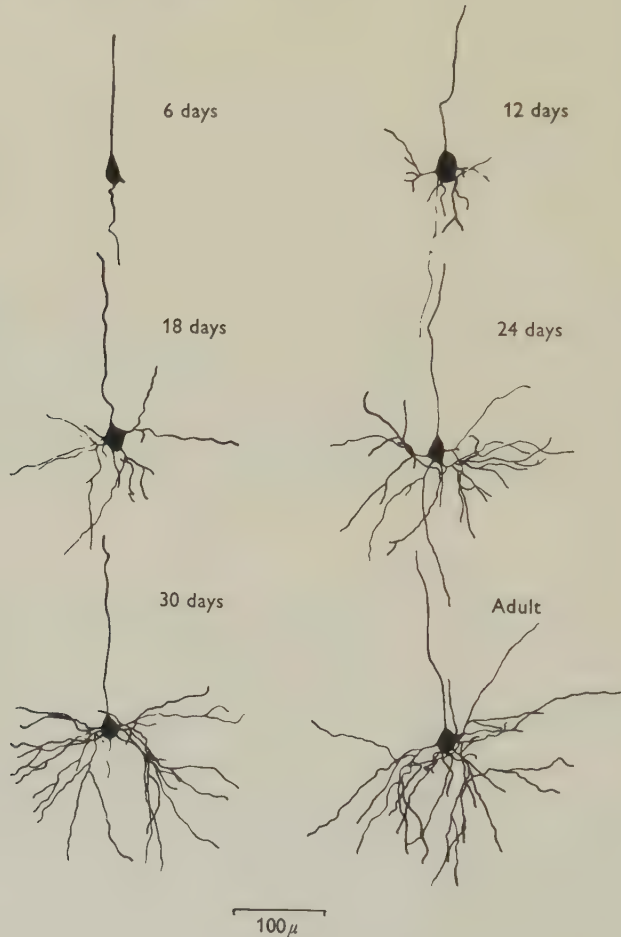


Fig. 2. Characteristic changes in appearance of pyramidal cells of the lamina ganglionaris from 6 days old to maturity.

One striking advance in development is seen in Golgi-Cox preparations, where many more neurones are impregnated. Considerable numbers of the apical dendrites now extend into layer 1, and numerous basal dendrites are present. Although these branch they do not extend far from the perikaryon, and there is, therefore, little if any overlap in the dendritic fields even of closely adjacent neurones (Fig. 2).

(iv) *The cerebral cortex from 18 days of age to maturity*

By the age of 18 days the cerebral cortex has assumed all the features characteristic of the adult, and changes between that age and maturity are quantitative rather than qualitative. All the laminae are now well marked, the cells of layer 5*b* are becoming conspicuously pyramidal in shape, and there is a marked increase in the extent of the dendritic fields, which now interlace with those of neighbouring neurones. Many such cells are now argyrophilic. The apical dendrites have increased in thickness and the majority extend into layer 1 and frequently branch. On a few of the apical dendrites, pedunculated bulbs can now be seen. The density of the axon network has markedly increased even to macroscopic inspection, particularly in the granular and infragranular layers, and fasciculi of radially coursing axons are now prominent in layer 5. These features become progressively more fully developed in the cortex of the 24- and 30-day-old rat, the most prominent changes being an increase in the complexity of dendritic ramification in layer 1 which is accompanied by a decrease in the number of axons in this region, an increase in the number of pedunculated bulbs and, apart from layer 1, a steadily increasing density of the axonal component of the neuropil.

(b) *Quantitative changes in cortical structure*

(i) *Cell/grey coefficient (Fig. 3)*

The cell/grey coefficient decreases most rapidly during the first days of life, whereafter the increment follows a slow and linear course until maturity. Not all laminae develop in the same way, however. Layer 1, which possesses very few identifiable neurones, shows very little change throughout the period of growth. The density of perikarya is greatest in the granular layer and remains so throughout the course of maturation. On the other hand, this density falls rapidly in both layers 3 and 5*a*, a feature which may to some extent be attributed to the rapid growth of apical dendrites of neurones in the subjacent layers which occurs between the 6th and 12th days of age. The somewhat later expansion of basal dendrites may account, to some extent, for the relatively delayed reduction in the cell/grey coefficient in layers 5*b* and 6*a*.

(ii) *Density of axons*

The density of axons does not increase at a uniform rate during cortical maturation. Only a small increase occurs during the first 7 days, but between the 6th and 18th day the increase is so rapid that at the end of this period the mean density is more than half the adult value (Fig. 4). Thereafter, the rate of increment is reduced, but by the 30th day falls short of the density in the fully mature cortex by a factor of only 15%. The period of greatest decrease in the cell/grey coefficient (birth-6 days) does not, therefore, coincide with that during which axons multiply most rapidly, although the points at which the curves for the packing density of perikarya and axon density begin to reach a plateau show a rough inverse correspondence (Figs. 3, 4).

The axon network does not develop at a uniform rate throughout the cortex, for although the fibre densities in the several laminae maintain approximately the

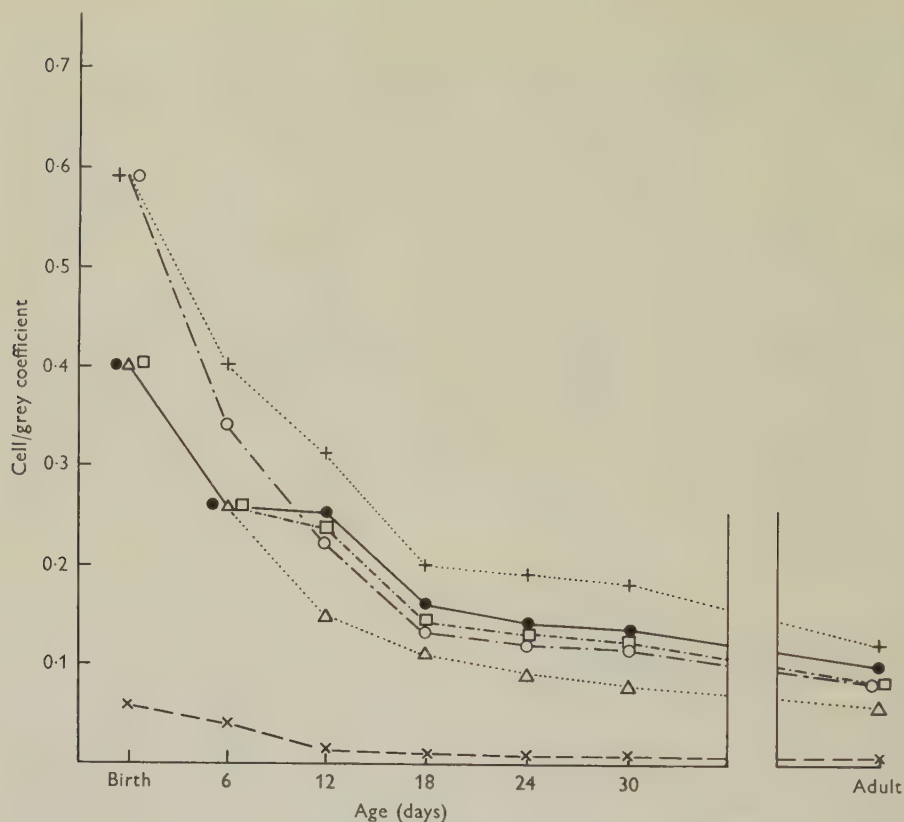


Fig. 3. Changes in the cell/grey coefficient in the several cortical laminae from birth to maturity. Key: layer 1, x---x; layer 3, O---O; layer 4, +.....+; layer 5a, Δ.....Δ; layer 5b, ●---●; layer 6, □---□.

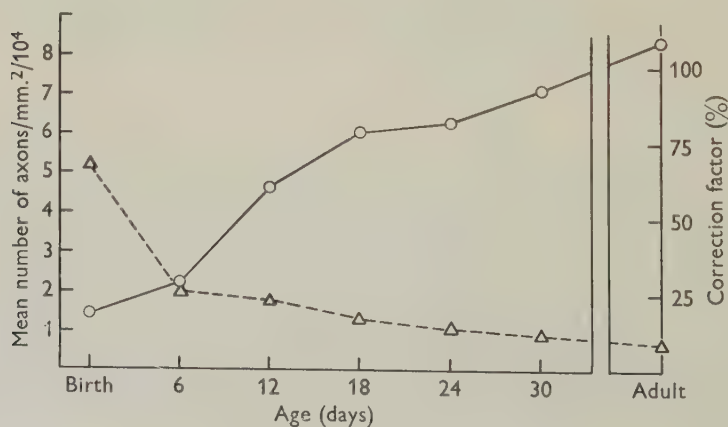


Fig. 4. Increase in number of cortical axons (O---O) and decrease in correction factor (i.e. the proportion of cortical tissue occupied by formed elements other than axons) (Δ---Δ) with advancing age.

same relationship with respect to each other from birth to maturity (Fig. 5) there are interlaminar differences in the periods during which growth is most rapid. In layers 3, 4, 5*b* and 6*a* the rate of increment is greatest between 6 and 18 days of age (Fig. 6), whereas in layer 1 the number of axons increases rapidly to reach a peak at 12 days old, after which there is a steady decrease in density. By contrast, layer 5*a* lags behind the remainder, a considerable proportion of its axons (32 %) appearing after the age of 30 days.

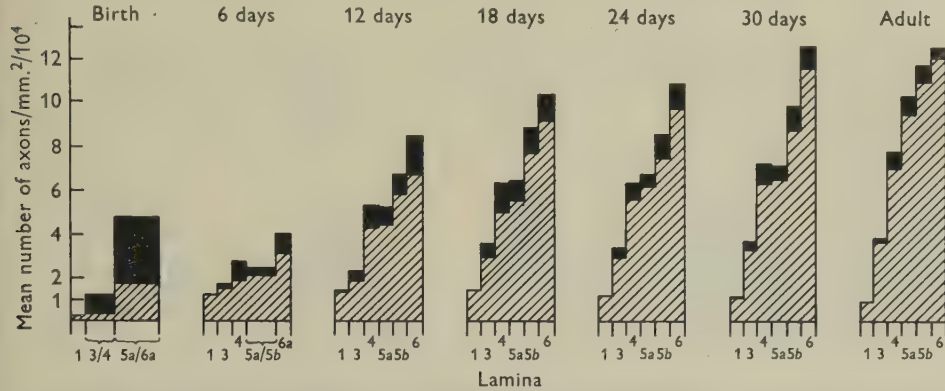


Fig. 5. Density of axons by laminae at successive ages. The hatched portion of each histogram gives the density in the cortex taken as a whole; the solid portion is the increment resulting from correction for the presence of formed elements other than axons, the total giving the axonal density within the neuropil.

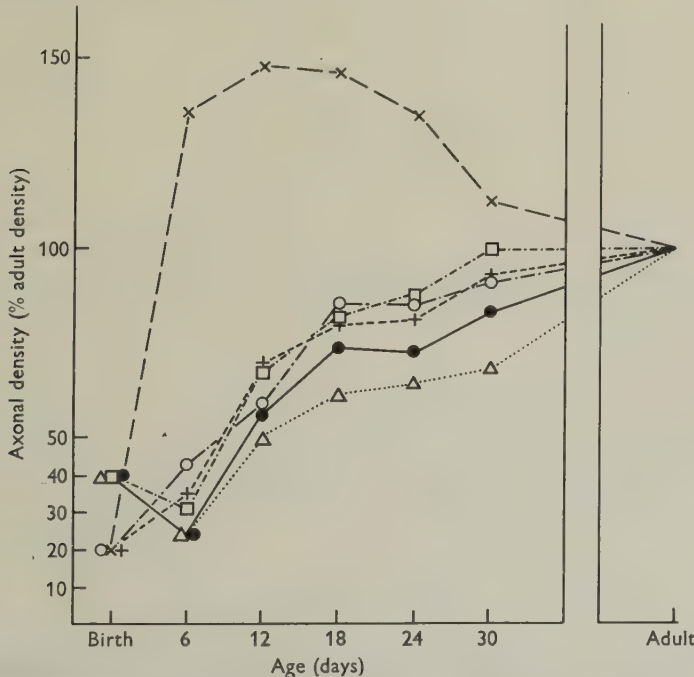


Fig. 6 Changes in axonal density with increasing age. Densities at each age are expressed as a percentage of their ultimate value in the adult. Key: layer 1, x---x; layer 3, O---O; layer 4, +---+; layer 5*a*, Δ....Δ; layer 5*b*, ●---●; layer 6, □---□.

(iii) *Dendritic fields*

Measurement was restricted to the dendritic fields of the pyramidal cells of the lamina ganglionaris. As described earlier, none of these neurones was impregnated at birth, and at 6 days old the few basal dendrites present extended but a negligible distance from the perikaryon. For this reason useful quantitative data are available only from the age of 12 days onwards.

Table 1. *Changes in pattern of dendritic fields formed by basal dendrites of pyramidal cells in the lamina ganglionaris from birth to maturity*

Property	Age					
	6 days	12 days	18 days	24 days	30 days	Adult
(1) Mean number of dendrites arising from perikaryon	0.6	5.4	5.2	5.3	5.3	5.2
(2) Mean number of branching sites	0.0	4.7	8.3	18.0	19.4	23.1
(3) Mean number of endings	0.6	10.1	13.5	23.3	24.7	28.3
(4) Branching index, i.e. ratio of (1) to (3)	1.0	1.9	2.6	4.4	4.7	5.5

Table 1 shows that the expansion of the dendritic field is characterized by two processes: an increase in the number of dendrites arising from the perikaryon and the peripheral branching of existing dendrites. The first of these is complete by the 12th day of age, at which time the number of primary basal dendrites has reached that characteristic of the adult. On the other hand, there is a steady increase in the amount of branching; the period of most rapid increment, as shown by changes in the branching index, occurs between the ages of 18 and 24 days, further increase in the dendritic field until maturity being relatively small.

The mode of distribution of dendrites at successive stages of development is illustrated by Fig. 7A, B, which shows the distribution of the points at which dendrites branch and end in relation to the perikaryon. At 12 days old the main weight of branching occurs between 18 and 36 μ from the centre of the perikaryon, and although the amount of branching taking place both at this distance and more peripherally increases steadily with advancing age the position of the peak remains unchanged. On the other hand, changes in the length of dendrites are shown by a shift of the peak of endings from the second zone (18–36 μ) in the case of the 12-day-old rat to about the 6th zone (90–108 μ) in the 30-day-old rat. Little change seems to occur in this respect between 30 days and maturity, but growth during this period is manifest by an increase in the number of dendrites which extend beyond the zone in which ending is maximal. This increase in the incidence of very long dendrites is further illustrated by the numbers which extend beyond the 180 μ limit of measurement. Such dendrites, which were first observed in the 24-day-old rat, have risen in number to a mean of 2.5 per cell in the adult.

The density of the dendritic field at increasing distance from the cell body is demonstrated by the families of curves shown in Figs. 7C and 8. In the former, the maximum density in the 12-day-old rat is seen to occur as close as 18 μ to the perikaryon and thence to decay rapidly. At later ages, however, not only is there an

overall increase in density at successive ages, but an initial predominance of branching over ending causes a rise in density to a peak which tends to move outwards as the animal grows older. In Fig. 8 the same data are expressed, as first described by Sholl (1953), in terms of the densities of dendrites emerging from the surfaces of a series of concentrically arranged spheres whose radii differ by

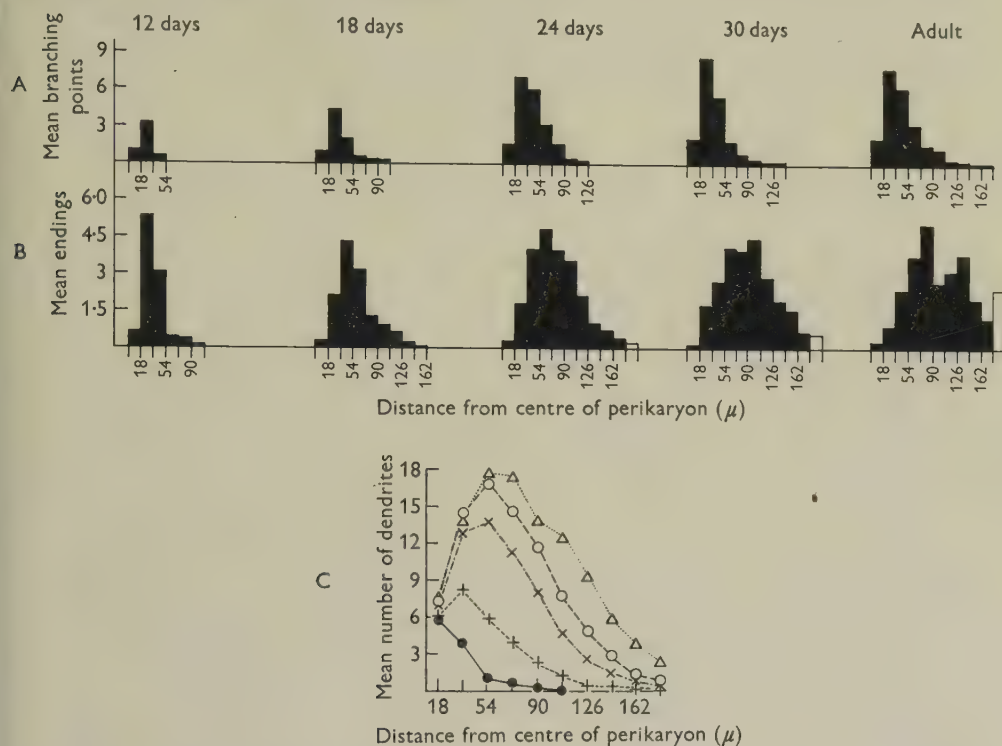


Fig. 7. Distribution of branching (A) and ending (B) points of basal dendrites in relation to the centre of the perikaryon. The white areas of the histogram for the distribution of endings show the increasing number of dendrites extending beyond the 180μ limit of estimation in the older animals. C gives the resultant dendritic densities at successive distances from the perikaryon. Key: 12 days old, \bullet — \bullet ; 18 days, +—+—+; 24 days, \times — \times — \times ; 30 days, \circ — \circ — \circ ; adult, \triangle \triangle .

a distance of 18μ . The obviously good fit of the calculated regression lines to the data shows that an exponential relationship of dendritic density and distance from the perikaryon is preserved throughout the course of development, the expansion of the dendritic field being expressed by a reduction in the size of the regression coefficient with advancing age.

DISCUSSION

Although there have been several previous accounts of the changes which occur during the maturation of the cerebral cortex (e.g. Paton, 1900; Ramón y Cajal, 1911; Sugita, 1917, 1918a-c; Tilney, 1933; Peters & Flexner, 1950) the present qualitative and quantitative findings, by emphasizing certain aspects of develop-

ment not touched upon by previous investigators, provide a more complete picture of the sequence of events than has so far been presented.

It is clear that, in the rat, only an elementary stage of differentiation has been reached at birth: the laminar arrangement of cells seen in the adult has not yet been attained, the perikarya are closely packed and few processes are present. Three phases: (i) the growth of axons, (ii) the growth of dendrites, and (iii) an increase in the spacing of perikarya, characterize the subsequent development of the neuropil but, while these overlap and are clearly inter-related, they do not coincide.

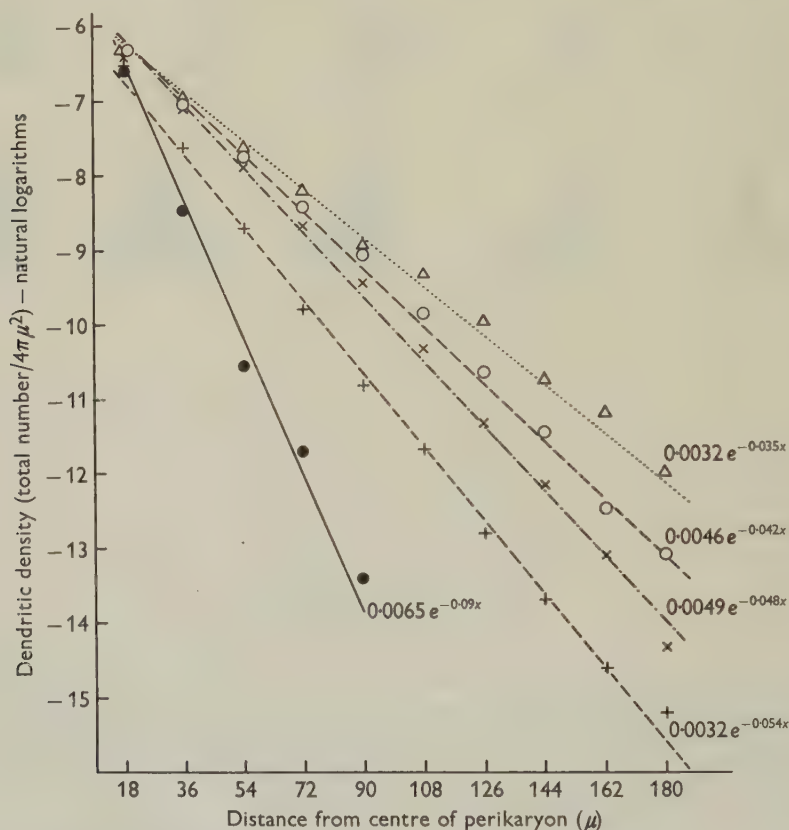


Fig. 8. Decay in density of dendritic fields with distance from the perikaryon at successive ages, together with parameters estimated for each regression. Key as for Fig. 7C.

The period of greatest reduction in cell density occurs during the first 6 postnatal days, in spite of the fact that during this period the total number of cells present is increasing as a result of mitotic activity (Allen, 1912) and the growth of cell processes is minimal. Thereafter, a markedly smaller rate of decrease in packing density is associated with the growth of cell processes, the period of most rapid increment in the density of axons (6–18 days) preceding that for the dendrites (18–24 days). Somewhat similar findings have been recorded by Peters & Flexner (1950), who showed that a major increase in the spacing of perikarya occurred between the 30th and 40th day of gestation in the guinea-pig, although even at the end of this

period very few cell processes could be identified by phase-contrast microscopy. It would thus appear that, judged by these criteria, the development of the cerebral cortex of the rat at birth has reached a stage similar to that of the guinea-pig at a gestation age of 30 days, and thereafter follows an essentially similar course.

These observations imply that the spacing of perikarya is not, as at first seemed likely, governed primarily by the growth of neuropil, but by the development of some histologically amorphous medium within which neural processes subsequently grow, branch and mature, and which they eventually come in part, at least, to replace. Several additional pieces of evidence support this view. First, in the deeper regions of the cortex there is actually a fall in the density of axons during the first 6 days of life, suggesting that the growth of the interstitial medium has exceeded that of the axons. Secondly, the size of the factor used to adjust the density of axons within the cortex as a whole to represent that within the amorphous background decreases very slowly at a time when cell processes are proliferating rapidly (Fig. 4) whereas, were the growth of processes the major cause of a reduced packing density of perikarya, it might be expected that the dimension of this factor would decrease in proportion to the increase in the density of cell processes. Finally, were the interstitial medium to remain constant in volume, the increased spacing of perikarya being solely due to proliferation of cell processes, then the density (as opposed to the total number) of axons in the neuropil would tend to remain constant, or even fall as axons thicken and myelinate. This, in the light of the results presented, is clearly not the case.

The nature of the interstitial medium within which the cell processes proliferate remains open to conjecture. The possibility that this is provided by neuroglial material cannot be excluded, but while no specific studies have been carried out to determine the amount of such material present, estimates of the volumes of such neuroglial elements as could be identified in silver-impregnated specimens gave no reason to suppose that a hyperplasia of this component could account for a decreasing cell/grey coefficient. There is, on the other hand, some evidence to suggest that the medium is extracellular, for in the guinea-pig there occurs, between the 30th and 40th days of gestation, a marked increase in chloride space (Flexner & Flexner, 1949) which coincides with an increase in the spacing of perikarya. Moreover, the extracellular space steadily decreases in volume during the time when cell processes are proliferating. These events are associated with a progressive increase in the intensity of staining by the periodic acid-Schiff reaction (Goodhead, 1957) which may therefore be interpreted as indicating an increasing concentration of the ground substance as processes grow within it.

These observations call into question the functional significance of the cell/grey coefficient. Nissl (1898), on the basis of studies made on the mole, dog, and man, first observed that cells were more closely packed in lower species than in higher, thus drawing attention to the possible importance of the neuropil separating the perikarya. This view was subsequently further developed by von Economo (1926) who, pointing to the greater possibility of neuronal inter-connexions in brains where cells were widely spaced, proposed that the cell/grey coefficient might be used as an index of the degree of organization, and hence presumably of functional capacity, of the cerebral cortex. This concept, whose significance has been discussed

by von Bonin (1948*b*), has been widely accepted. The present results show that the coefficient can be applied as an ontogenetic index only with caution, for although it shows a steady decrease from birth to maturity, this decrease now appears to be due to factors other than the growth of neuropil, while its major portion precedes the period of maximal dendritic growth. Furthermore, the value of the coefficient as a phylogenetic index is equally open to doubt and is in any case vitiated by inadequate and inconsistent data. The present measurements for the rat, although designed to provide a basis for comparison between ages rather than absolute criteria, conform almost exactly with the earlier estimates of Eayrs & Taylor (1951), for the 24-day-old rat and are of the same order as, though slightly smaller than, those of Peters & Flexner (1950) who used the statistical sampling method of Chalkley (1943) in their study of the foetal guinea-pig. The mean cell/grey coefficient found for the adult rat (9 %) is, as might be expected, larger than that proposed by von Economo for man (3.7 %), which, in the light of Agduhr's (1941) criticism, must be regarded as being on the high side. Von Bonin (1952) and Shariff (1953), on the other hand, using similar methods, derived cell/grey coefficients for eulaminate cortex of 21 % for man and 36 % for *Tarsius*, while Sholl (1953) has computed a value of 25 % for the striate cortex of the cat, all these estimates far exceeding those obtained for both rat and guinea-pig and the earlier and more widely accepted data for man. Such inconsistencies presumably have their origin in such variables as the mode of preparation of the tissues, thickness of section, the region of cortex examined and, particularly where the method of Chalkley (1943) is used to measure the coefficient, in the depth of focus of the optical system and the subjective judgement of various observers as to what is, or is not, in focus. Until such factors are adequately standardized there can be little value in attempting to use the cell/grey coefficient as a comparative index of cerebral organization (for further critical discussion see Haug, 1956).

A more valuable criterion of functional capacity, though technically more laborious to acquire, would appear to be the probability of interaction between axons and dendrites within the neuropil. Sholl (1956) has recently drawn attention to the importance of the quantitative aspects of cortical organization in relation to function and has used concepts such as those of Cragg & Temperley (1954), Beurle (1954) and Sholl & Uttley (1953), which are based on the apparently statistical arrangement of connexions within the neuropil, to stress the view that the excitation of neurones may be governed more by the probability of their interaction under varying conditions than through the medium of precisely organized circuits. Such probabilities will clearly depend, among other things, on the densities of both the axons and dendrites, and since throughout the course of development the density of the basilar dendritic field associated with each pyramidal neurone decays exponentially with distance from the centre of the perikaryon it is possible, by applying the formulae of Uttley (1955), to derive the changes in probability of connexion between neurones which occur during cortical maturation in the rat. Approximate values obtained in this way are plotted in Fig. 9, and it is perhaps of interest to note that the placing reflex, which depends on the integrity of the region of cortex studied (Brooks, 1933) can first be elicited at about 17 days of age when the probability of interaction may be estimated, by interpolation, at 100

functional 'contacts' per neurone. The maturation of behaviour is retarded in the hypothyroid individual (Eayrs & Lishman, 1955), and it may be significant that at the time when the placing reaction first appears (24 days) the factor of connectivity, derived from the data of Eayrs (1955), proves to be of the same order (95 connexions) as in the normal. Such observations indicate a useful field of study, but clearly insufficient work has so far been undertaken to assess the value of the correlations between anatomically determined connectivity and behavioural capacity.

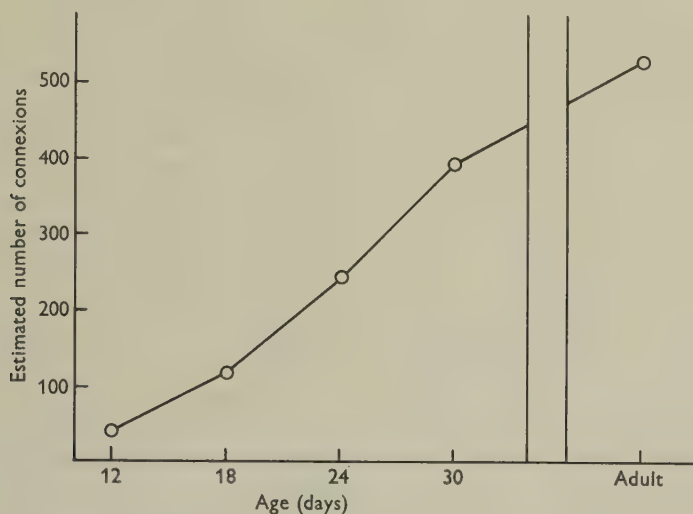


Fig. 9. Changes in estimated connectivity resulting from the maturation of neuropil.

The data provided by present experiment do, however, serve to throw further light on the effects of thyroid deficiency on cortical maturation. Earlier, it has been shown that in the 24-day-old hypothyroid rat the growth of the axon network in layer 4 is more severely retarded than in other layers and that the decay of the dendritic field departs from the normal exponential pattern. The form of the curves showing the mode of increase in axon density in the several laminae (Fig. 6) and the fact that the dendritic field decays exponentially throughout development provide no evidence to suggest that the cortex of the hypothyroid rat resembles that of a younger animal. It must therefore be inferred that the changes arising as a result of thyroid deficiency represent a distortion rather than a retardation of growth.

SUMMARY

1. A qualitative and quantitative study has been made, using Nissl, silver and Golgi-Cox preparations, of the changes which take place in the sensorimotor cortex of the rat during the course of development.

2. At birth the neurones are very closely packed throughout the cortical thickness, and individual laminae cannot readily be distinguished. An increase in cell size and spacing is accompanied by laminar differentiation, layers 3 and 4 becoming distinguishable at 6 days old and layer 5a at 12 days.

3. Few axons are present at birth, and during the early stages of development these run a predominantly tangential course. Bundles of radially orientated axons, presumably specific thalamic afferents, do not appear until between 12 and 18 days of age. Very few dendrites appear until after the 6th day of life, but after the 12th day growth and multiplication by branching are rapid. By the 18th day cortical structure has attained its adult characteristics and thereafter changes are quantitative rather than qualitative.

4. The periods of most rapid change in the cell/grey coefficient, and in the density of axons and dendrites do not coincide. The cell/grey coefficient decreases most rapidly between birth and 6 days old; the density of axons increases maximally between the ages of 6 and 18 days and that of dendrites between 18 and 24 days.

5. Changes in axonal density in the several cortical laminae do not coincide. Increments in layers 3, 4, 5*b* and 6*a* follow much the same time course, but the density of axons in layer 1 reaches a maximum at about 12 days old and thereafter decreases, while that in layer 5*a* is delayed by comparison with the remainder.

6. The mean number of dendrites arising from the perikaryon has reached the adult figure as early as the 12th day of age. Subsequent development of the dendritic field is marked by a peripheral extension of dendrites and by an increased amount of branching. Branching remains maximal at a distance of 18–36 μ from the centre of the perikaryon throughout the course of development. On the other hand, the number of dendritic endings reaches a peak which lies between 18 and 36 μ in the 12-day-old rat and is steadily shifted away from the perikaryon to a distance of about 108 μ in the adult. The density of the dendritic field preserves an exponential pattern of decay throughout the course of its development.

7. These findings are discussed from a functional point of view and with reference to the somewhat different pattern of development which occurs in hypothyroidism.

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AFFERENT FIBRES TO THE DORSO-MEDIAL THALAMIC NUCLEUS IN THE CAT

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INTRODUCTION

The frontal cortex has been shown to be reciprocally connected to the dorso-medial thalamic nucleus (see, for example, Monakow, 1895; Le Gros Clark & Boggon, 1933; Walker, 1938; Waller, 1940; Rose & Woolsey, 1948; Meyer 1949; Auer, 1956) and the further connexions of this nucleus are clearly essential to an understanding of the frontal cortex.

Rioch (1931), Le Gros Clark (1932) and Walker (1938) have suggested the hypothalamus and the periventricular fibre system as a possible source of afferent fibres to the dorso-medial nucleus, but Le Gros Clark & Meyer (1950) have added that the anatomical details of this projection are far from complete, while Walker states that an exact knowledge of the afferent fibres to the dorso-medial nucleus is lacking.

During a previous study of the rat's hypothalamus (Guillery, 1957) it was found that some of the lesions gave rise to degeneration of fairly coarse fibres in the dorso-medial nucleus. The origin of this degeneration could not be strictly localized on the material then available, but by using cats it has been possible to obtain finer localization of the lesions and to show that the fibres in question come from the septum and from the mid-brain. They do not come from the hypothalamus as had been expected originally.

MATERIALS AND METHODS

Twenty-eight adult cats were used. In eighteen of these, lesions were placed in the septum, hypothalamus and mid-brain and the remaining ten were used as unoperated control material. The operations were carried out under Nembutal (sodium pentobarbitone) anaesthesia, and precautions were taken against infection. In most of the animals a ventral approach to the central nervous system was used, going through the soft palate and the base of the skull (see Markowitz, 1949). This ventral approach allows reasonably accurate placement of lesions and has the advantage of leaving the dorsal parts of the brain undamaged.

The cats were firmly held in a head holder and the hypophysis exposed. The gland was left *in situ* and was used as a landmark for guiding the electrode to the selected site. The electrode was a stainless steel needle, the last 5 mm. of which were 100 μ in diameter. The shaft was insulated with several coats of varnish and only the tip was left bare. The electrode was held in a firm clamp and the lesions were made by passing a current of 0.5–1.0 mA. for 15–30 sec. with the electrode positive. Each electrode placement was used for obtaining records of blood pressure changes before the lesions were made. This part of the work was done by Dr B. G. Cragg and will form a separate report.

Eight to fifteen days after the operation the animals were killed under Nembutal

anaesthesia. They were perfused through the heart with saline, followed by 10% neutralized formol saline. The brains were left for about 3 months in the formalin and blocks about 7 mm. across were then embedded in carbowax and sectioned at 20 μ . Every 10th section was mounted serially on gelatinized slides and stained with cresyl violet. Other sections were stained by the Nauta method (Nauta & Gyax, 1954) and arranged in serial order by reference to the Nissl series. In each block as many sections were stained as were necessary to give a clear serial picture of the degenerating pathways. In carbowax embedded material the Nauta staining can be done at considerable intervals of time. The sections can be left in covered trays for a number of weeks and the Nauta stain shows no deterioration. The majority of blocks were cut parasagittally.

As in the rat, the Nauta reaction is slightly altered by carbowax embedding. In the cat it has been found advantageous to use only 10 min. in 0.25% phosphomolybdic acid followed by about 5 min. in 0.025% potassium permanganate. This gives a clean dark staining of degenerating fibres and a lighter staining of normal fibres in the regions investigated. It is probable that different times would prove more suitable for the more caudal parts of the central nervous system. For good results it has been found advantageous to dissolve the carbowax away from the section in warm water (45°–50° C.) and to store the sections in formol saline overnight before staining.

Control material and criteria for identifying degeneration have been used as previously (Guillery, 1957). The terms sparse, moderate, dense and heavy again refer to increasing grades of density of degeneration. These terms cannot be given any precise quantitative significance; they are used as a rough relative guide to the size of the projections that have been found.

RESULTS

Afferent fibres have been traced to the dorso-medial nucleus from three sources. One group of coarse fibres comes from the ventro-medial parts of the mid-brain, a second comes from the medial septal nucleus and the nucleus of the diagonal band, and a third group, made up of finer fibres, probably comes from the lateral septal nucleus and the nucleus accumbens.

(1) *The mesencephalic projection*

A number of the rats that were used for a previous investigation of hypothalamic connexions showed moderate to dense degeneration in the dorso-medial nucleus. In some of these animals (rats 286 and 289, see Guillery, 1957) the lesion included the anterior parts of the hypothalamic medial forebrain bundle but in others (330, 331, 346, 347) the relevant lesion appeared to be in the mid-brain. Mesencephalic lesions were placed in cats 389, 429, 430 and 432 to study these fibres further.

The lesions in cats 429, 430 and 432 are shown in Text-fig. 1, and Text-fig. 2 shows the degeneration that passes from the lesion to the dorso-medial thalamic nucleus in cat 430. In this animal the fibres ascend along the habenulo-peduncular tract, lying in the most anterior part of the tract. Pl. 1, figs. 1 and 5 show these fibres lying close to the normal, pale staining tract. The fibres enter the postero-dorsal part of the dorso-

medial nucleus and radiate out anteriorly and ventrally. There is moderate degeneration in the greater part of the nucleus, but the most postero-ventral, anterior, medial and lateral portions of the nucleus are virtually clear of degeneration. The pattern of the degeneration is similar in the other three animals, although it is less dense than in cat 430.

The mesencephalic component does not give a dense contribution to the dorso-medial pericellular plexus in any of the cats. Comparison of the lesions suggests that the fibres arise from or pass through the ventral tegmenta larea of Tsai, in relation to the caudal end of the medial forebrain bundle, before they become associated with the habenulo-peduncular tract. An origin from the interpeduncular nucleus itself cannot be completely excluded but, on the basis of the available material, an origin from the regions lateral or posterior to the nucleus is more probable.



Text-fig. 1. The lesions in cats 429, 430 and 432 shown on a frontal reconstruction. The extent of the lesions has been mapped by reference to nuclear groups and fibre tracts. The outlines for this figure and for Text-figs. 3 and 8 are taken from Jasper & Ajmone-Marsan (1955) by courtesy of the authors. Horizontal shading, cat 429; vertical shading, cat 430; diagonal shading, cat 432.

(2) *The anterior projection*

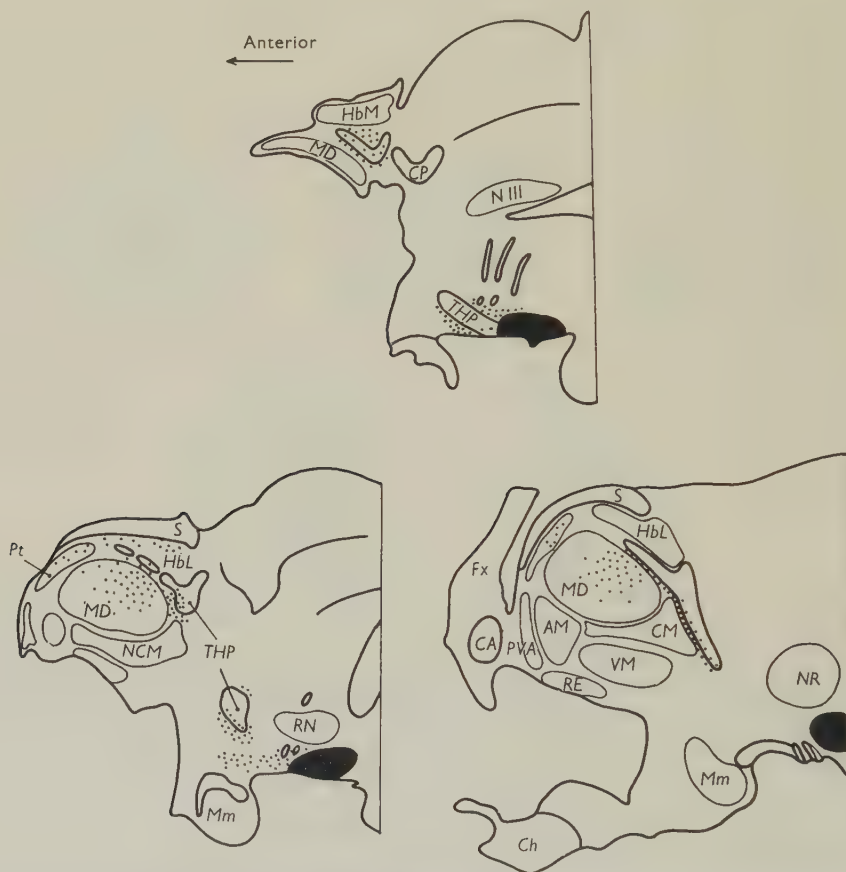
(a) *Coarse fibres*

A large number of relatively coarse fibres pass from the medial septal nucleus and the nucleus of the diagonal band to the dorso-medial thalamic nucleus. These fibres arise from a continuous cell column that lies amongst the anterior parts of the medial forebrain bundle. They will be called the coarse anterior projection in the following account and will be divided into a direct and an indirect group according to their route to the dorso-medial nucleus.

Both groups are clearly shown in cat 403. In this animal the lesion on the right side includes only the ventral part of the medial septal nucleus and a small, adjacent portion of the diagonal band. On the left the lesion also includes the anterior part

of the hypothalamic medial forebrain bundle and a part of the internal capsule and entopeduncular nucleus (Text-fig. 3).

On the right, coarse degenerating fibres can be seen passing ventrally from the lesion into the diagonal band (Text-fig. 4 B). Text-fig. 4 shows that the further course of these fibres is extremely tortuous. They form the indirect group. The fibres first turn laterally into the horizontal limb of the diagonal band (C) and then leave this by turning posteriorly into the lateral preoptic area (C). They join the anterior part of the hypothalamic medial forebrain bundle but leave this after a short distance by turning dorso-medially opposite the optic tract (C). They pass towards the posterior

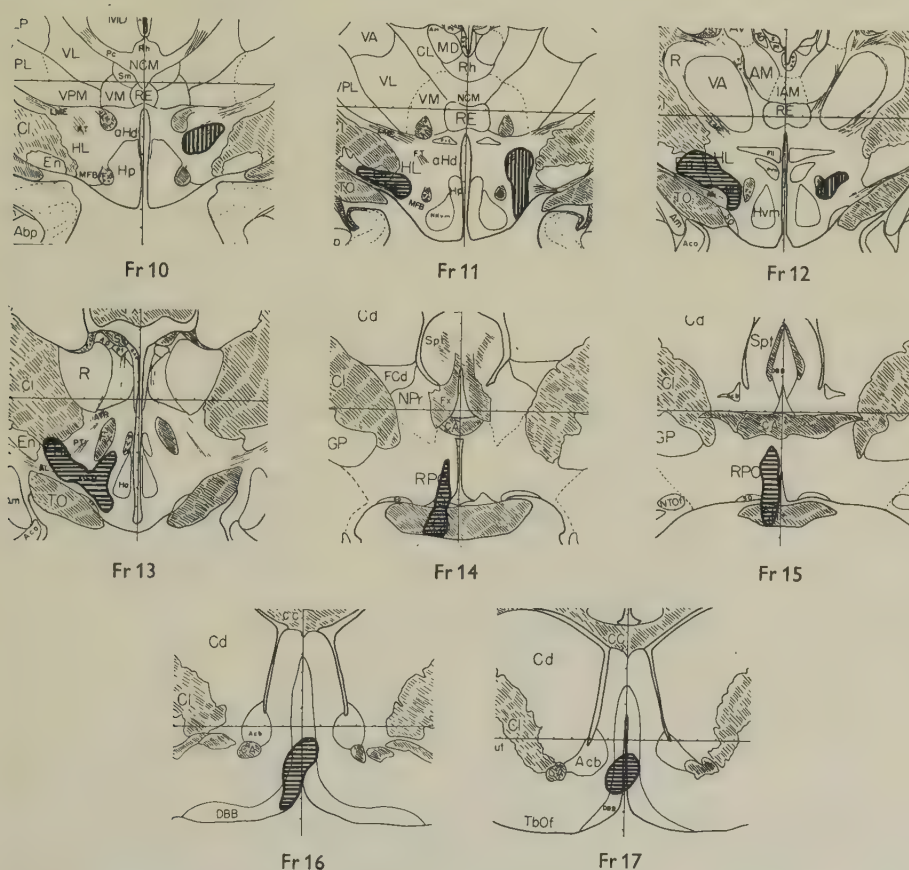


Text-fig. 2. Cat 430. Degeneration in the mesencephalic projection to the dorso-medial nucleus. Outline drawings of three sections from the parasagittal series showing the distribution of the relevant degeneration. The most medial section is shown at the top of the figure.

aspect of the stria medullaris (B) and through the parataenial nucleus towards the habenular nuclei (A). They then pass through, and close to, the lateral habenular nucleus and end by radiating out into the postero-dorsal parts of the dorso-medial nucleus from around the origin of the habenulo-peduncular tract (A). There is only sparse degeneration in the stria medullaris (Pl. 1, fig. 2) and sparse to moderate

degeneration in the lateral habenular nucleus. Within the dorso-medial nucleus moderate degeneration is directed anteriorly and becomes sparser as the anterior pole of the nucleus is approached. The postero-ventral quarter of the nucleus is free of degeneration and so is the lateral third.

On the left the same group of fibres can be traced from the lesion to the dorso-medial nucleus, but in addition there is a second group of fibres which takes a less tortuous course. This 'direct' group leaves the medial forebrain bundle posterior



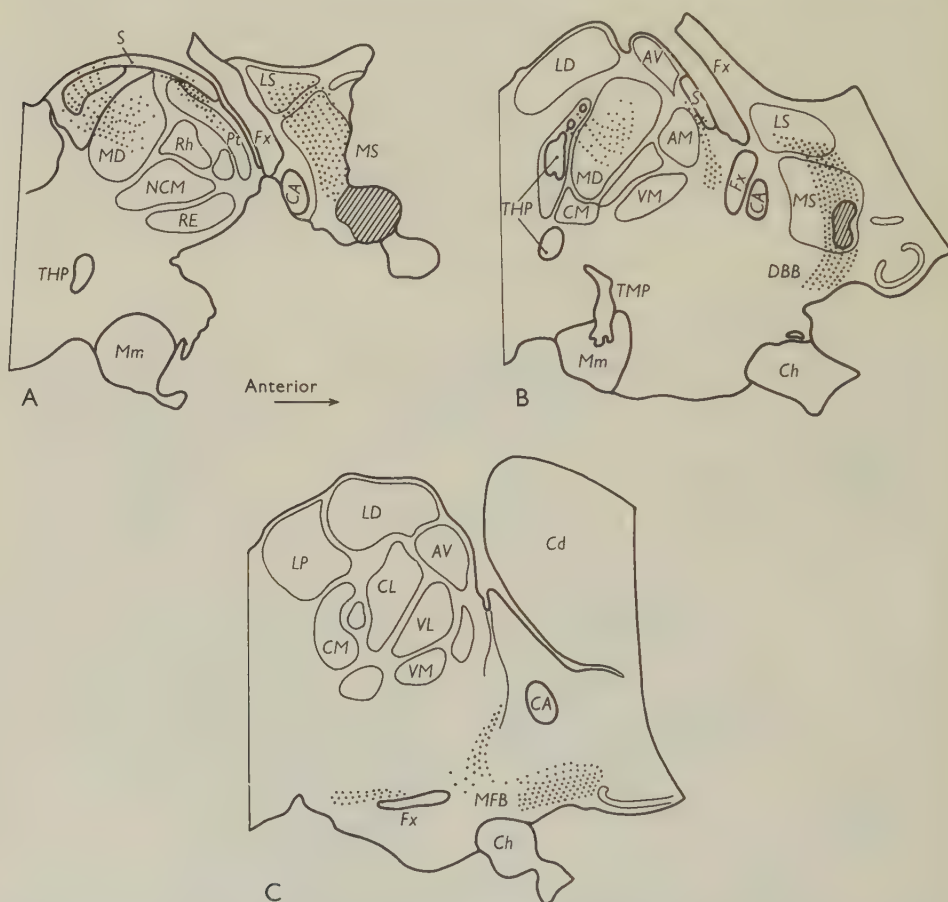
Text-fig. 3. The lesions in cats 402 and 403. For notes see Text-fig. 1.
Horizontal shading, cat 403; vertical shading, cat 402.

to the first group and passes dorso-medially and slightly posteriorly, lateral to the post-commissural fornix and medial to the mamillo-thalamic tract. The fibres pass in small bundles through the internal medullary lamina and enter the antero-ventral parts of the dorso-medial nucleus.

In cat 412 these direct fibres have been damaged as they lie ventro-medial to the mamillo-thalamic tract (see also Pl. 1, fig. 4) and the more rostral, indirect fibres have been spared. Text-fig. 5 shows that the direct fibres distribute to the posterior and ventral parts of the dorso-medial nucleus, that their field of distribution over-

laps that of the indirect fibres to a large extent and that neither of the two groups supplies a significant number of fibres to the lateral quarter or third of the dorso-medial nucleus. The immediate periventricular region is virtually free of coarse-fibred degeneration.

The coarse direct and indirect fibres to the dorso-medial nucleus have been damaged unilaterally in cats 405, 407, 409, 421, 424, 435 and 447. These animals confirm the course and the distribution given above and, together with cats 434, 446

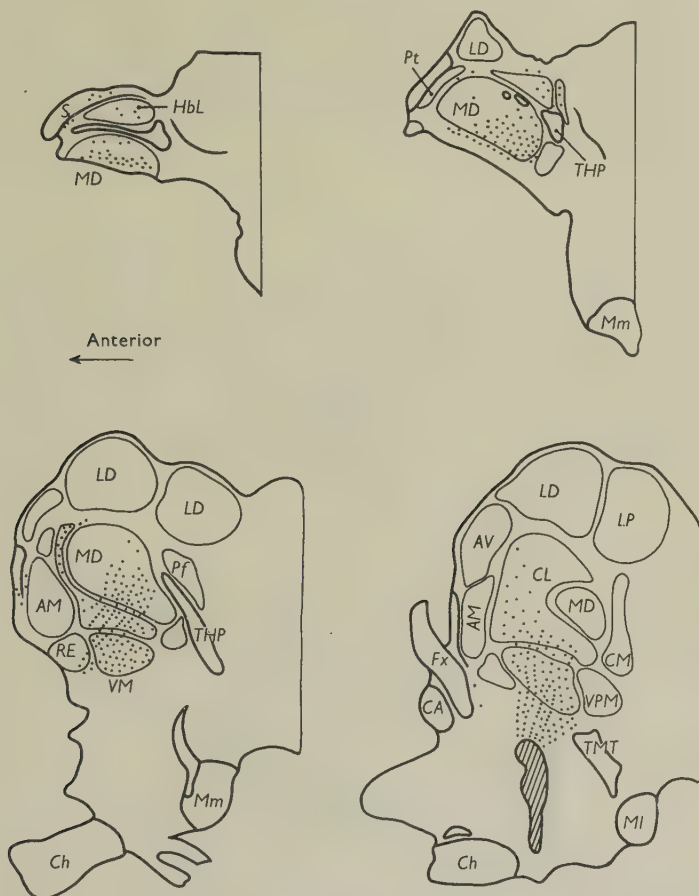


Text-fig. 4. Cat 403R. Degeneration in the 'indirect' fibre group. Outline drawings of three sections from the parasagittal series showing the distribution of the degeneration. The most medial section is shown at the top left part of the figure.

and 449L and R in which the hemispheres have been damaged anterior to the septum they show that the origin of the fibres lies amongst the anterior parts of the medial forebrain bundle.

Since the coarse anterior projection is uncrossed in all the animals with unilateral lesions the indirect fibres in cat 403R must arise from or pass through the ventral part of the medial septal nucleus. If they are to pass through the septum they must

arise either from the hippocampus or the medial wall of the hemisphere anterior to the septum. The hippocampus can be excluded since there is no evidence for any projection from the fornix system to the dorso-medial nucleus (Sprague & Meyer, 1950; Nauta, 1956; Guillery, 1956; Hamlyn, 1958).



Text-fig. 5. Cat 412. Degeneration in the 'direct' fibre group. Outline drawings of four sections from the parasagittal series showing the distribution of the relevant degeneration. The most medial section is shown at the top left part of the figure.



Text-fig. 6. The lesions in cats 446 and 449L. The outlines have been traced from photographs of the medial aspects of the hemispheres.

The medial wall of the hemisphere has been damaged in four hemispheres. Text-fig. 6 shows the lesion in cats 446 and 449L. In cat 446 the lesion was made by an electrode that passed close to the mid-line and through the chiasma. The electrolytic scar lies anterior to the medial septal nucleus and in the antero-ventral part of the lateral septal nucleus, reaching from the mid-line to within a short distance of the lateral ventricle. In cat 449L the lesion was made by passing a small knife from the region of the cruciate sulcus towards the olfactory tubercle. The ventral part of the lesion (see Text-fig. 6) extends between the mid-line and the lateral ventricle. Dorsally cortical areas 4, 5, 6, 8 and 32 of Winkler & Potter (1914) have been partially damaged or undercut.



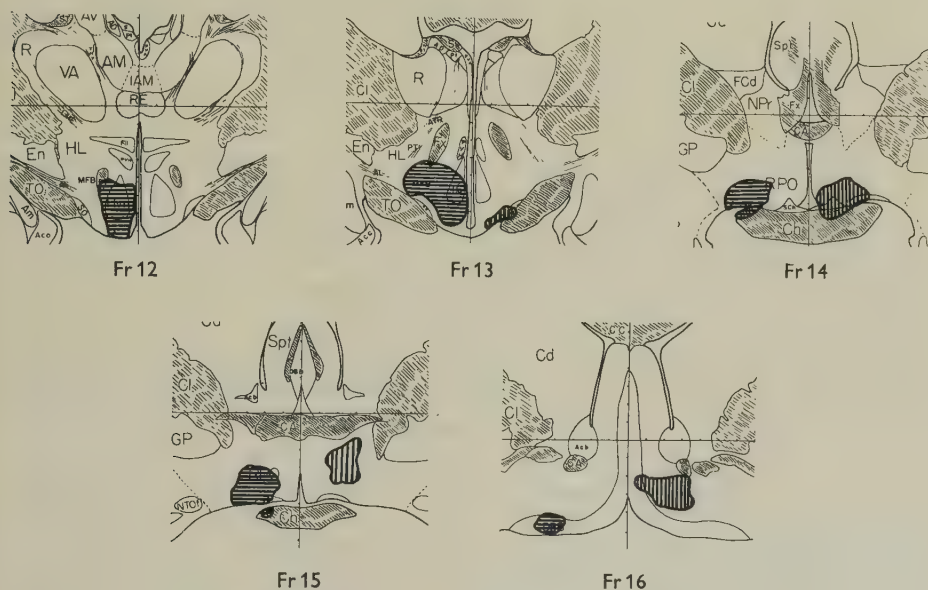
Text-fig. 7. The lesion in cat 434. Outline drawing of two sections from the parasagittal series. The more medial section is at the top.

Since the direct and the indirect fibres to the dorso-medial nucleus are free from degeneration in these animals the fibres cannot enter the dorsal parts of the septum from anteriorly. An entry into the ventral parts of the septum from the olfactory tubercle or nucleus accumbens* is unlikely from the position of the lesion in cat 403R. It is excluded by cat 434 in which the lesion lies immediately anterior to the diagonal band and spares only a small medial part of the nucleus accumbens (Text-fig. 7). In this animal there is no degeneration in either the direct or the indirect coarse anterior projection to the dorso-medial thalamic nucleus.

* 'Nucleus accumbens' is used here in accordance with the description given by Fox (1940).

No part of the anterior coarse projection therefore arises from the medial parts of the hemisphere immediately anterior to the diagonal band and medial septal nucleus. The indirect component must arise in the medial septal nucleus and adjacent diagonal band. The origin of the direct component needs further discussion.

In cat 435 the lesion is similar to that shown for cat 434 except that the anterior part of the lesion extends further laterally into the caudate nucleus and a small posterior part of the lesion includes the medial portion of the horizontal limb of the diagonal band. In this animal there is degeneration in the direct and indirect projections to the dorso-medial nucleus, showing that some of the direct fibres arise in the diagonal band.



Text-fig. 8. The lesions in cats 421 and 424. For notes see Text-fig. 1.
Horizontal shading, cat 421; vertical shading, cat 424.

The anterior, pre-hypothalamic portion of the medial forebrain bundle (lateral pre-optic region and nucleus of the diagonal band) had also been damaged in cats 405, 424 (Text-fig. 8) and 447, and in each of these animals there is degeneration in the direct as well as the indirect fibres. Many of the direct fibres thus arise anterior to the hypothalamus. None of the present material gives clear evidence of a hypothalamic contribution to the dorso-medial nucleus. Cat 402 (Text-fig. 3), in which the posterior part of the lateral hypothalamus was damaged and in which there is no degeneration in the dorso-medial nucleus, excludes the posterior part of the hypothalamic medial forebrain bundle as a possible origin. In cat 421 (Text-fig. 8) it was possible to trace the direct fibres to the anterior, subcommissural, part of the lesion but not to the posterior, hypothalamic part. The anterior part of the lateral hypothalamus and the medial hypothalamus have not been selectively damaged by any of the lesions and they cannot be excluded as a possible additional source of afferent fibres to the

dorso-medial nucleus. However, at present there is no evidence that such afferents arise in the hypothalamus.

It can therefore be concluded that the direct fibres arise from the anterior parts of the medial forebrain bundle, immediately posterior to the origin of the indirect fibres. They degenerate after lesions confined to the medial parts of the medial forebrain bundle, and run mainly in the more medial parts of the bundle. Fibres also run to the dorso-medial nucleus from the frontal cortex (e.g. Auer, 1956; Meyer, 1949). The majority of these fibres run further laterally than the direct fibres. However, a few join the medial forebrain bundle and course with the direct fibres. Cat 449L and R and cat 459, in which considerable parts of the frontal cortex were damaged, show that the antero-grade degeneration to the dorso-medial nucleus is relatively light and that only a few of these cortico-thalamic fibres join the medial forebrain bundle and the direct group of fibres. The cortico-thalamic fibres can form only a minority of the direct fibres.

(b) *Fine fibres*

In some of the animals with anterior lesions there was, in addition to the coarse-fibred projection described above, a finer projection passing to the dorso-medial nucleus (cats 405, 407, 424, 434, 435). This can be distinguished by a number of features. It is a bilateral projection, it only passes to the medial parts of the dorso-medial nucleus and it produces degeneration that contrasts well with that shown by the previous group (Pl. 2, figs. 8, 9, 10 and 11).

The fine fibres show degeneration in cat 434 in which the coarse fibres are normal. In cats 403R and L (Text-fig. 3) and cats 412 and 421 (Text-fig. 8) the coarse anterior fibres show degeneration while the fine fibres are normal.

In cat 434 (Text-fig. 7) the fine fibres can be traced from the damaged nucleus accumbens to the lateral subcommissural region, where they lie dorsal to the position occupied by the coarse fibres.* They turn sharply medially and posteriorly just posterior to the level of the anterior commissure and enter the antero-ventral part of the medial thalamus (nucleus reuniens). The degeneration is continuous through the inter-antero-medial nucleus and the nucleus centralis medialis into the medial third of the dorso-medial nucleus.

The distribution of the lesions in the other animals confirms this course but does not give a precise localization for the origin of the fine fibres. They may arise from the nucleus accumbens or they may enter this nucleus from the lateral septal nucleus, the olfactory tubercle, the caudate nucleus or the fronto-medial cortex.

DISCUSSION

(1) *Methods*

The method that has been used in the present investigation has been used previously in the rat (Guillery, 1957), and has been discussed by a number of observers (Glees & Nauta, 1955; Evans & Hamlyn, 1956; Nauta, 1957; Cragg & Hamlyn, 1959). Only some of the problems that arise from the use of the method will be discussed here.

* Here they lie in the same position as do the fine fibres that ascend to the lateral septal nucleus in the rat (Guillery, 1957).

(a) *Negative findings*

Several of the operated animals have been used because they failed to show degeneration in one or more of the fibre systems afferent to the dorso-medial nucleus. In these 'negative' animals there has always been well-stained degeneration in some part of the forebrain. The negative finding was only taken as useful when it was clear that the staining method was successful for the particular animal under investigation.

(b) *Fibre size*

The fibres afferent to the dorso-medial nucleus have been classified as 'coarse' or 'fine' largely on the basis of the degeneration picture (cf. Pl. 2, figs. 6–11). In general it has been found that fibre tracts having coarser fibres produce coarser degeneration granules (e.g. a comparison between the mamillary peduncle and the fornix degeneration in the mamillary bodies). Pl. 2, fig. 6, shows degeneration in the mamillo-thalamic tract of cat 389. The mesencephalic fibres to the dorso-medial nucleus were also damaged in this animal and Pl. 2, fig. 7, shows that these fibres are coarser than the mamillo-thalamic fibres and that their degeneration is more advanced.

Pl. 2, figs. 8–11, also show a comparison between the coarse and the fine degeneration in two animals (cats 407 and 409). From these figures it appears that the exact degeneration appearances vary between animals, but that there is always a marked difference between the coarse group and the fine group. While the coarse fibres are always slightly thicker than the mamillo-thalamic fibres, the fine fibres are generally thinner. The axon diameter of the mamillo-thalamic fibres, excluding myelin sheath, on normal Holmes material lies in the region of 1μ .

(c) *Survival times*

The survival times that have been used for the cats varied between 8 and 15 days. Most of the animals were allowed 10–12 days post-operative survival. Nauta (1957) has found that the optimal survival time for the cat is 7–10 days as opposed to about 5 days in the rat. Two cats with 7-day survival periods showed practically no degeneration after hypothalamic lesions and in cat 389 in which the survival time was 8 days some of the tracts are still in a very early stage of degeneration (Pl. 2, fig. 6). The rats that showed degeneration in the dorso-medial thalamic nucleus had been given between 3 and 5 days post-operative survival.

These times suggest that the degeneration found in the dorso-medial nucleus is not a retrograde change. On the Nauta sections there has been no clear evidence of retrograde fibre degeneration in any of the tracts that have been divided (e.g. superior cerebellar peduncle, mamillary peduncle, habenulo-peduncular tract, medial lemniscus). Retrograde cellular changes have only been found in cats 449 and 459. In these animals considerable areas of cortex were damaged 11 and 10 days before death and the retrograde changes can be clearly recognized in the Nissl preparations of the dorso-medial nucleus. The Nauta sections show a dense, fine, granular deposit, which is co-extensive with the areas of retrograde cell degeneration (Pl. 2, figs. 12, 13). This Nauta deposit cannot be mistaken for either the mesencephalic or for the coarse rostral projection to the dorso-medial nucleus, which always form a characteristic

pericellular plexus. The contrast with the fine fibred group is less clear cut. However, the fine fibred group is probably afferent to the dorso-medial nucleus since it is possible to trace the whole of the course of these degenerating fibres to the thalamus and since there has been no sign of retrograde cellular change in any of the relevant animals.

(2) *The connexions of the dorso-medial nucleus*

Le Gros Clark & Boggon (1933) have described Marchi degeneration in the cat following a lesion ventro-medial to the anterior thalamic nuclei (their cat 6). They found degeneration passing to the parataenial and dorso-medial thalamic nuclei and to the stria medullaris. It is probable that their lesion included the anterior coarse as well as the fine fibred projection to the dorso-medial nucleus.

Nauta (1958*a*) has described a lesion in the lateral pre-optic region of the cat which gave rise to degeneration in the dorso-medial nucleus. He does not give a further account of this degeneration, but it must correspond, at least in part, to the projection that has been found here.

Nauta & Valenstein (1958) have also described a projection to the dorso-medial nucleus in the monkey. This comes from the amygdaloid nuclei, and passes through the sublenticular region and inferior thalamic peduncle to the magno-cellular part of the nucleus. Nauta (1958*b*) has found a related pathway in the cat. In this animal the substantia innominata receives afferent fibres from the amygdaloid nuclei and sends fibres to the dorso-medial nucleus. A number of the present lesions have included the substantia innominata and a part of the projection that has been described here, particularly of the fine fibred group, may well correspond to that found by Nauta. However, the position of some of the present lesions (403R, 407, 435) which lie close to the mid-line and rostral to the anterior commissure shows that there is an additional projection to the dorso-medial nucleus from the medial septal nucleus and the diagonal band.

Fortuyn *et al.* (1959) have also found a projection to the dorso-medial nucleus from the basal parts of the hemisphere, including the septum and the amygdaloid nuclei. It appears that the dorso-medial nucleus receives a considerable number of afferent impulses either directly or indirectly from these two parts of the telencephalon.

Papez (1932), in his account of the normal thalamus of the nine-banded armadillo, describes the coarse plexus of the parafascicular nucleus as invading the dorso-medial nucleus. It is probable that this appearance is produced by the mesencephalic fibres that ascend along the habenulo-peduncular tract.

Other workers have described afferent fibres to the dorso-medial nucleus coming from other thalamic nuclei (Rioch, 1931; Le Gros Clark, 1932; Papez, 1932), from the periventricular system (Rioch, 1931; Le Gros Clark, 1932; Walker, 1938) and from the frontal cortex. Only the fibres from the frontal cortex have been traced on experimental material.

Mettler (1935, 1947), Meyer (1949) and Auer (1956) have described the fibres that pass from the frontal cortex to the dorso-medial nucleus. Meyer, working on human material, has added that the amount of degeneration is always surprisingly small.

In the present investigation it has been possible to trace three separate groups of fibres afferent to the dorso-medial nucleus. One arises from the ventral tegmental area of Tsai or from regions postero-lateral to it. It is closely related to the caudal end of the medial forebrain bundle. The second, a fine fibred projection, probably arises from the lateral septal nucleus but may arise from adjacent parts of the forebrain. The cells of origin of these two groups have not been localized precisely. The third group arises from the rostral parts of the medial forebrain bundle, including the ventral portion of the medial septal nucleus and the nucleus of the diagonal band.

Daitz & Powell (1954) have shown that most of the large cells of the medial septal nucleus and some of the large cells of the diagonal band nucleus undergo retrograde degeneration following section of the fimbria. These cells therefore send their axons into the fimbria and it is improbable that they also send axons to the dorso-medial nucleus. It is possible that the dorso-medial projection arises from the smaller cells that survived the fimbrial lesion. An alternative hypothesis would be that in the part of the medial septal nucleus close to the fimbria the majority of cells project to the fimbria and that in the horizontal limb of the diagonal band the majority of cells project to the thalamus. In the intermediate region both types of projection may be present and the region of overlap would then lie largely in the ventral part of the medial septal nucleus and in the vertical limb of the diagonal band nucleus.

The dorso-medial nucleus thus receives afferent fibres from the anterior and posterior parts of the medial forebrain bundle, from the frontal cortex and, either directly or indirectly, from the amygdaloid nuclei. The relationships of the last two projections to the fibres that have been described here need further investigation. Some of the cortico-thalamic fibres contribute to the direct component, and the fine fibred projection may well correspond to the amygdaloid projection. The possibility that the hypothalamus projects to the dorso-medial nucleus is not excluded by the present material. The postero-medial parts of the hypothalamus in particular need further investigation. However, at present there is no good evidence that the hypothalamus sends any fibres to the dorso-medial nucleus.

All the afferent fibres that have been described here form a projection to the medial three-quarters of the dorso-medial nucleus. It has never been possible to see more than sparse degeneration in the lateral quarter of the nucleus. It has been shown that in primates the medial, magnocellular part of the dorso-medial nucleus projects to the orbital cortex, while the larger, lateral part of the nucleus projects to the dorsal areas of the frontal lobe (e.g. Walker, 1940; Meyer, Beck & McLardy, 1947). Waller (1940) and Rose & Woolsey (1948) have studied the frontal projection of the dorso-medial nucleus in the cat and both have found a medio-lateral organization of the projection. However, Rose & Woolsey point out the dangers of attempting to homologize the relevant cortical fields of primates with those of other mammals. At present no clear homologies can be claimed for the subdivisions that have been described in the dorso-medial nucleus in the cat and in primates. It is probable that comparative studies of the afferent fibres to the dorso-medial nucleus will prove particularly useful in establishing such homologies.

The localization of the afferent fibres that have been described here does not correspond closely to Rioch's (1929) subdivision of the nucleus. The fine fibres project to the medial third of the nucleus and the coarse fibres to the medial three-quarters.

The mesencephalic fibres have a more lateral distribution than the anterior fibres and the direct fibres have a more posterior distribution than the indirect fibres. There may thus be some antero-posterior organization of the projection from the anterior parts of the medial forebrain bundle but it must be stressed that there is considerable overlap between the various groups.

(3) *General considerations*

A preliminary investigation of the medial forebrain bundle (Guillery, 1957, and unpublished observations) shows that it forms a mixed system of cell bodies and ascending and descending fibres. Anteriorly it links with the hippocampus and posteriorly with the ventro-medial mid-brain and peri-aqueductal grey. The medial septal nucleus, the nucleus of the diagonal band, the lateral pre-optic region and lateral hypothalamus all form a part of this system (see also Nauta, 1958*a* for a description of this system). Nerve cells lie scattered among the fibres throughout the medial forebrain bundle and they send their processes for variable lengths along the bundle. It is probable that many of the individual neurons have ascending and descending processes. At the level of the hypothalamus the medial forebrain bundle forms a number of transverse connexions with the medial hypothalamic nuclei. It is thus an important afferent and efferent pathway for the hypothalamus.

The fibres that have been described here appear to provide a direct pathway from the medial forebrain bundle to the dorso-medial nucleus. The coarse anterior projection comes from the anterior part of the medial forebrain bundle (medial septal nucleus and diagonal band), while the mesencephalic fibres, which closely resemble the rostral fibres in their termination, probably come from the posterior part of the medial forebrain bundle (ventral tegmental area or a more caudal region). In this way the dorso-medial nucleus is able to receive the impulses that pass to and from the hypothalamus. It does not appear to receive impulses directly from the hypothalamus.

It may be useful to regard the dorso-medial nucleus as a mechanism that records the changes produced in the medial forebrain bundle by hypothalamic activity. On the basis of its known connexions the nucleus is in a position to compare hypothalamic inputs with hypothalamic outputs and thus to record the physiological adequacy of hypothalamic responses rather than the responses themselves.

Recently the effects of damage in the anterior and dorso-medial thalamic nuclei have been studied (Schreiner, Rioch, Pechtel & Masserman, 1953; Brierley & Beck, 1958). Two points relevant to such investigations arise from the present findings. The majority of lesions in the anterior thalamic nuclei are likely to destroy the anterior part of the afferent projection to the dorso-medial nucleus. The interpretation of the differential effects of such lesions thus becomes extremely complex. However, a clear comparison between these two thalamic cell groups would be of considerable value. The anterior thalamic nuclei receive a topographically well-organized pattern of impulses that comes from the hippocampus and peri-aqueductal grey via the mamillary bodies (see Powell, Guillery & Cowan, 1957, for references). Since the hippocampus is linked to the anterior end of the medial forebrain bundle and the peri-aqueductal grey to its posterior end the afferent projection to the anterior thalamus resembles that to the dorso-medial nucleus in many

respects. However, it is less closely related to the hypothalamus, and, grossly, it shows a more complex organization than does the projection to the dorso-medial nucleus.

A close topographical association between the coarse fibred projections to the dorso-medial nucleus and the habenular system has been found throughout this investigation. It was surprising that the fibres to the dorso-medial nucleus should lie in such close relation to a phylogenetically old part of the diencephalon. The close relationship is likely to be more than a mechanical convenience, and it is probable that useful information about the dorso-medial nucleus will come not only from studies of primate fibre connexions but also from more detailed studies of much simpler brains.

SUMMARY

1. The afferent fibres to the cat's dorso-medial thalamic nucleus have been traced by the method of Nauta & Gyax (1954).

2. Fibres afferent to the medial three-quarters of the dorso-medial nucleus arise in the medial septal nucleus, the nucleus of the diagonal band and in the ventro-medial mid-brain.

3. Fibres afferent to the medial third of the dorso-medial nucleus, finer than the previous group, arise from the nucleus accumbens or from regions adjacent to this nucleus.

4. It has not been possible to trace afferent fibres to the lateral quarter of the dorso-medial nucleus.

5. There is no evidence that fibres from the hypothalamus pass directly to the dorso-medial nucleus. The fibres that enter the dorso-medial nucleus all come from nuclear groups that lie on the afferent or efferent pathways of the hypothalamus.

I wish to thank Prof. J. Z. Young for his advice and encouragement and to thank Miss B. Shirra for technical assistance; Mr J. Armstrong for the microphotographs, and Miss J. de Vere for the drawings.

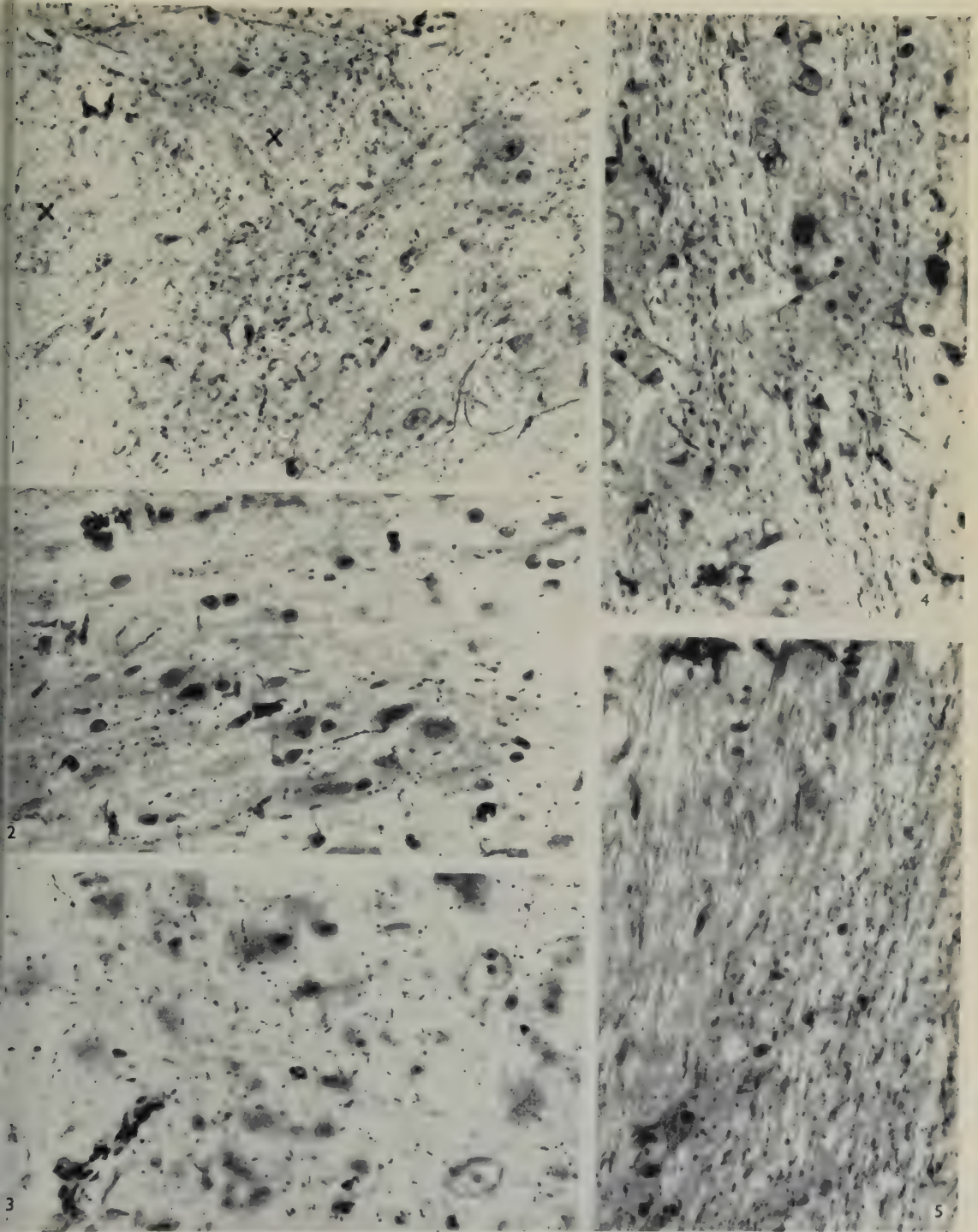
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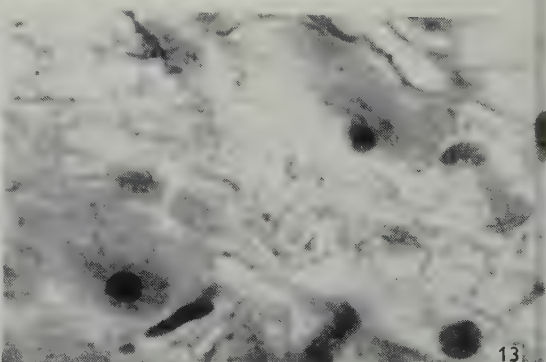
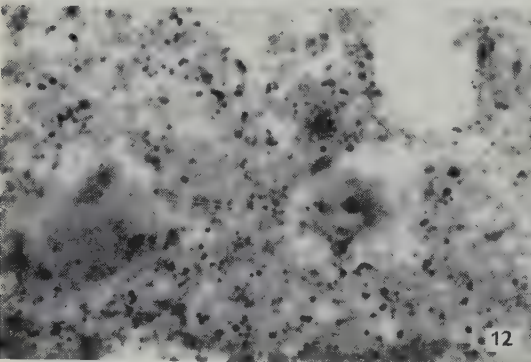
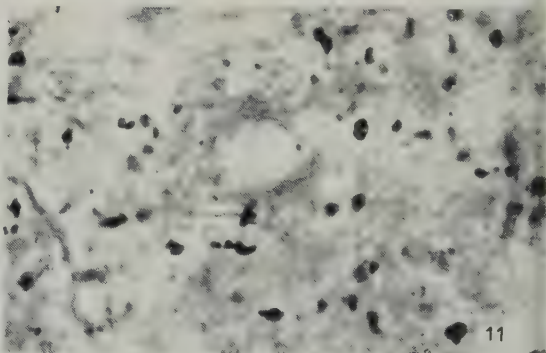
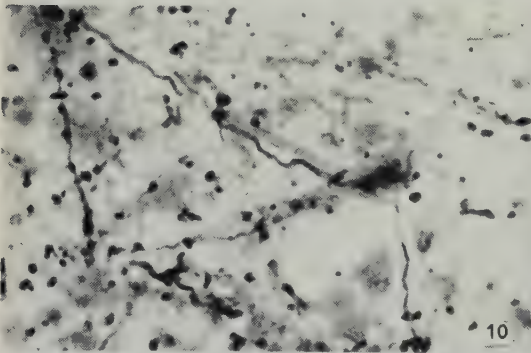
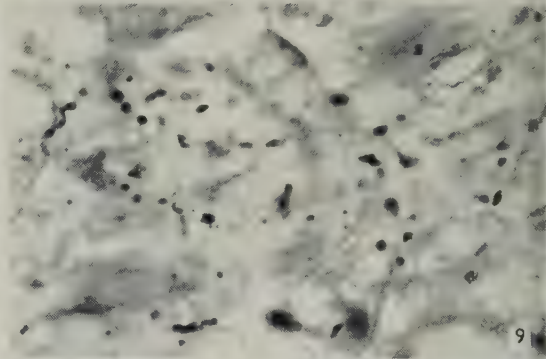
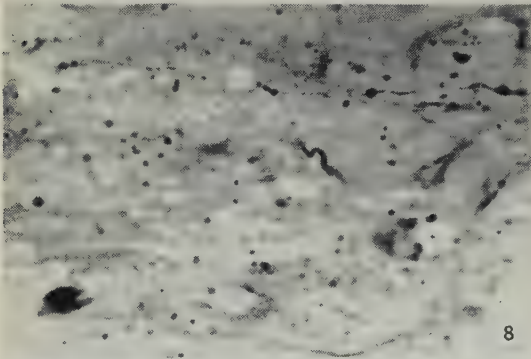
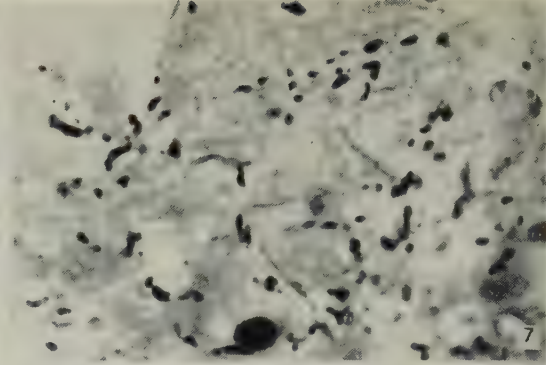
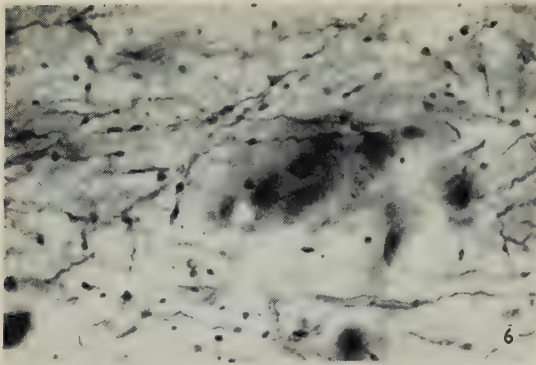
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KEY TO ABBREVIATIONS

<i>Acb</i>	Nucleus accumbens	<i>Cd</i>	Caudate nucleus
<i>aHd</i>	Dorsal hypothalamic area	<i>Ch</i>	Optic chiasma
<i>AM</i>	Antero-medial nucleus	<i>CI</i>	Internal capsule
<i>AV</i>	Antero-ventral nucleus	<i>CL</i>	Nucleus centralis lateralis
<i>CA</i>	Anterior commissure	<i>CM</i>	Nucleus centrum medianum



(Facing p. 418)



GUILLERY—AFFERENT FIBRES TO DORSO-MEDIAL THALAMIC NUCLEUS IN CAT

<i>CP</i>	Posterior commissure	<i>NCM</i>	Nucleus centralis medialis
<i>CS</i>	Superior colliculus	<i>NHvM</i>	Ventro-medial hypothalamic nucleus
<i>DBB</i>	Diagonal band	<i>NR</i>	Red nucleus
<i>DBc</i>	Decussation of brachium conjunctivum	<i>Ped</i>	Cerebral peduncle
<i>EN</i>	Entopeduncular nucleus	<i>Pf</i>	Parafascicular nucleus
<i>Fx</i>	Fornix	<i>Pt</i>	Parataenial nucleus
<i>GC</i>	Central grey	<i>PVA</i>	Paraventricular nucleus
<i>Hg</i>	Anterior hypothalamus	<i>R</i>	Reticular nucleus
<i>HbL</i>	Lateral habenular nucleus	<i>RE</i>	Nucleus reuniens
<i>HbM</i>	Medial habenular nucleus	<i>Rh</i>	Nucleus rhomboideus
<i>HL</i>	Lateral hypothalamus	<i>RPO</i>	Pre-optic region
<i>Hvm</i>	Ventro-medial hypothalamic nucleus	<i>S</i>	Stria medullaris
<i>HP</i>	Posterior hypothalamus	<i>SN</i>	Substantia nigra
<i>IP</i>	Interpeduncular nucleus	<i>TbOf</i>	Olfactory tubercle
<i>LD</i>	Nucleus lateralis dorsalis	<i>THP</i>	Habenulo-peduncular tract
<i>LP</i>	Nucleus lateralis posterior	<i>TMP</i>	Principal mamillary tract
<i>LS</i>	Lateral septal nucleus	<i>TMT</i>	Mamillo-thalamic tract
<i>MD</i>	Dorso-medial thalamic nucleus	<i>TO</i>	Optic tract
<i>MFB</i>	Medial forebrain bundle	<i>VA</i>	Nucleus ventralis anterior
<i>MI</i>	Lateral mamillary nucleus	<i>VL</i>	Nucleus ventralis lateralis
<i>Mm</i>	Medial mamillary nucleus	<i>VM</i>	Ventro-medial thalamic nucleus
<i>MS</i>	Medial septal nucleus	<i>VPM</i>	Nucleus ventralis postero-medialis
<i>N III</i>	Third nerve nucleus		

EXPLANATION OF PLATES

All the material for the figures has been prepared by the method of Nauta & Gyga (1954).

PLATE 1

- Fig. 1. Degeneration in the mesencephalic projection to the dorso-medial nucleus. The fibres are shown entering the postero-dorsal parts of the nucleus close to the normal bundles of the habenulo-peduncular tract which have been marked X. Cat 429. 11 days' survival. Parasagittal section. $\times 400$.
- Fig. 2. Degeneration in the 'indirect' projection to the dorso-medial nucleus. The degenerating fibres are shown in the parataenial nucleus. The relatively normal stria medullaris occupies the upper part of the picture. Cat 403R. 15 days' survival. Parasagittal section. $\times 400$.
- Fig. 3. Degeneration in the 'indirect' projection to the dorso-medial nucleus. The central part of the dorso-medial nucleus. Cat 403. 15 days' survival. Parasagittal section. $\times 400$.
- Fig. 4. Degeneration in the 'direct' projection to the dorso-medial nucleus. The bundles of degenerating fibres are shown on their way to the antero-ventral aspect of the nucleus. Cat 424. 10 days' survival. Parasagittal section. $\times 400$.
- Fig. 5. Degeneration in the mesencephalic projection to the dorso-medial nucleus. The degenerating fibres are shown close to the normal habenulo-peduncular tract, which occupies the upper, left part of the picture. Cat 389. 8 days' survival. Parasagittal section. $\times 400$.

PLATE 2

- Figs. 6, 7. These are taken from the same section. Fig. 6 shows degeneration in the anterior thalamus and fig. 7 shows degeneration in the coarse fibres to the dorso-medial nucleus. Cat 389. 8 days' survival. Both $\times 1100$.
- Figs. 8, 9. These are taken from the same section. Fig. 8 shows degeneration in the fine fibred projection to the dorso-medial nucleus and fig. 9 shows degeneration in the coarse fibred projections. Cat 407. 12 days' survival. Both $\times 1100$.
- Figs. 10, 11. These are taken from the same section and again show degeneration in the fine (fig. 10) and the coarse (fig. 11) projections to the dorso-medial nucleus. Cat 409. 10 days' survival. Both $\times 1100$.
- Figs. 12, 13. These are taken from the same section. Fig. 12 shows retrograde degeneration in the dorso-medial nucleus and fig. 13 shows the normal antero-dorsal nucleus. Cat 459. 10 days' survival. Both $\times 1100$.

In figures 6-11 and 13 a number of pale staining normal fibres can be seen.

AXO-SOMATIC AND AXO-DENDRITIC SYNAPSES OF THE CEREBRAL CORTEX: AN ELECTRON MICROSCOPE STUDY

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1. INTRODUCTION

The method of electron microscopy is, of course, of extreme value for the study of the cytology and membrane relations of neurons and neuroglia. However, the study of the nervous system is to an important degree a study of the connexions of neuronal processes. This is at present an impossible task for the electron microscope (EM), not only because of the impracticability of serially sectioning a useful volume of tissue, but because the cell processes of grey matter twist and turn in all directions. This results in oblique sectioning, causing membranes to become invisible (see Williams & Kallman, 1955). Nevertheless, electron microscopy should prove invaluable in this connexion in assessing the accuracy of information obtained by the light microscopist, who can then continue with his silver, Nissl, Golgi, Weigert and other techniques with added confidence.

Various regions of the mammalian central nervous system have been examined with the EM, e.g. the spinal cord (Wyckoff & Young, 1956), the thalamus (Fernández-Morán, 1955) and the ventral cochlear nucleus (de Robertis, 1956) (see also Sjöstrand, 1956). Palade (1954) has studied the cerebellum and medulla and Palay (1956, 1958) has studied the cerebrum, cerebellum, medulla and neurohypophysis. Horstmann & Meves (1958) have studied the brains of fishes and reptiles. All have reported that the pre-synaptic process contains numerous synaptic vesicles and often mitochondria and the contact region between pre- and post-synaptic components shows localized density increases and thickenings. Synapses in the cerebral cortex in particular have received little attention. Palay (1956) mentioned, without illustrating, that cortical synapses are in general similar to those elsewhere in the central nervous system, except that the pre-synaptic processes are smaller and contain fewer mitochondria. Luse (1956) studied the cerebral cortex and other regions, but did not describe synapses in the cortex. Schultz, Maynard & Pease (1957) made a more detailed study of the cerebral cortex and described structures containing 'synaptic vesicles' and thickened contacting-membranes. From their observations (see Discussion) they doubted that these features could be considered specific criteria for the identification of synapses.

From observations given in this report it is suggested that the criteria are still valid. Structural differences between axo-somatic and certain axo-dendritic synapses are described. The latter type show marked densities associated with the thickened region of the post-synaptic membrane, an increased spacing where the synaptic membranes are thickened, and the occurrence of an extracellular band of material between those regions of the synaptic membranes. Finally, the spines of dendrites

are shown to be sites of synaptic contact (Gray, 1959*b*). The term 'synapse' has been used to label morphologically specialized contacts between neuronal processes. These are presumed to be regions of transmission, but the precise site of transmission within these contacts is unknown.

2. METHODS

The visual area of the occipital cortex of adult rats (Lashley, 1944) has been used throughout. No attempt has been made to study specific layers, but layer 1 has been given least attention. Three methods have been used:

(a) A rat was anaesthetized with ether and the skull roof and dura removed from the visual cortex. Vertical slices were obtained by using four razor blades held parallel and 0.5 mm. apart in a clasp. The slices were transferred to 1 % osmium tetroxide in Ringer's solution, buffered at pH 7.4 with veronal acetate and maintained at about 5° C. Fixation was continued for 4 hr. with continuous agitation. After dehydration with ethanol, the slices were 'stained' with 1 % phosphotungstic acid in absolute alcohol for 3 hr., again with continuous agitation. Araldite embedding (Glauert & Glauert, 1958) was used and the sections were examined with a Siemens Elmiskop 1*b* electron microscope.

(b) As (a), using osmium tetroxide for fixation but omitting immersion in phosphotungstic acid. This showed no structural differences from method (a), but the material showed very poor contrast.

(c) As (a), but 0.6 % KMnO_4 (Luft, 1956) was used in place of 1 % osmium tetroxide but again the phosphotungstic acid was omitted. Observations made by this method are described in §5.

In the following sections, method (a) has been used, except where otherwise stated.

3. NEURONAL PERIKARYA, DENDRITES AND AXONS

Profiles of neuronal perikarya (Pl. 2, fig. 8; Pl. 3, fig. 11) are recognized by size, shape, the cisternae and granules of the endoplasmic reticulum, the shape and density of the nucleus and other features (see Palay & Palade, 1955; Luse, 1956; Schultz, Maynard & Pease, 1957).

Palay (1956) described tubular structures running longitudinally in dendrites of the central nervous system and regarded them as part of the endoplasmic reticulum. Schultz *et al.* (1957) observed structures which appeared to them as dots in cross-sections of dendrites of the cerebral cortex and named them neural filaments. They made no mention of the tubular nature of these structures. Palay's description is confirmed here (Gray, 1959*a*).

The dendrite tubules are about 200 Å in diameter and are shown in longitudinal section in Pl. 1, fig. 3 and Pl. 3, fig. 10. Often they run parallel, but in places they approach each other and possibly anastomose (Pl. 2, fig. 5). In cross-sections the tubules are seen as regularly spaced ring profiles (Pl. 1, fig. 1, Pl. 5, fig. 19). When the dendrite branches or changes direction in the plane of section, the tubules can be seen cut in all planes (Pl. 5, fig. 17). The group at (a) are normal to the plane of section, those at (b) are cut obliquely, and those at (c) run longitudinally. It can be seen that the rings are most dense; the oblique profiles (b) appear as two short, less dense lines and

the longitudinally orientated tubules (c) appear the least dense. The effect, of course, depends on differences in the amount of electron-scattering material lying in the axis of the beam (Williams & Kallman, 1955). The result is that when dendrites are sectioned obliquely or longitudinally their tubules may be almost or completely invisible (Pl. 1, fig. 4, b). This is especially obvious in the low contrast preparations obtained when phosphotungstic acid was omitted from the technique. Such processes can appear 'clear' or 'empty' and become indistinguishable from certain neuroglial processes.

Myelinated axons are easily recognizable and nodes are observed occasionally (Pl. 6, fig. 24). Tubules can sometimes be observed in the preterminal regions of small diameter unmyelinated axons (Palay, 1956) and it is sometimes impossible to distinguish them from small dendrites (see §7). Systems of tubules have not so far been observed in processes, which could be traced to neuroglial cell-bodies (see Luse, 1956; Schultz *et al.* 1957; Gray, 1959c).

4. SYNAPSES OF THE CEREBRAL CORTEX

General. In sections pre-synaptic processes (diameters up to $1.5\ \mu$) contain numerous ring profiles 200–600 Å. in diameter (Pl. 1, figs. 1, 3; Pl. 2, figs. 5, 6)—the so-called synaptic vesicles. Mitochondria are sometimes present (Pl. 2, fig. 5; Pl. 4, figs. 12, 13). Examination of sections of 300 pre-synaptic processes showed that 47 % contained no mitochondrial profiles, 43 % contained one, 8 % contained two, and 1 % contained four, the maximum observed. Serial sections are, of course, necessary to determine the mitochondrial content in any given pre-synaptic process, but such figures can be legitimately used to make a comparison with populations of pre-synaptic processes in electron micrographs of other regions of the central nervous system, for in these processes mitochondria are said to occur frequently (Palay, 1956; Schultz *et al.* 1957).

The post-synaptic component can be perikaryon, dendrite trunk or dendrite spine (see below). Often the post-synaptic component appears in the neuropil as a round, conical or oval-shaped profile. Many of these processes might be oblique sections through spines (§6).

Regions of the apposed membranes of the pre- and post-synaptic processes show thickenings and increased densities as noted by previous workers (Pl. 1, figs. 1–4). The synaptic vesicles occur in the pre-synaptic process and appear in clusters near these thickenings (Palay, 1956; Schultz *et al.* 1957).

These thickened regions show special properties. Pl. 5, fig. 18, shows a part of the damaged cut edge of a slice of cortex. The cell processes have become widely separated in places and their membranes are ruptured. The thickened regions, however, remain firmly attached. Three such regions are shown, the denser and thicker membrane of the post-synaptic process (see below) is seen attached to a pre-synaptic process in each case.

One very obvious feature of cortical synapses is that in certain contacts with dendrite trunks or their spines a high proportion of the length over which the membranes are apposed shows a thickening and increased density. Also the thickening and density is much more pronounced in the post- than the pre-synaptic membrane (see below). Such synapses are designated type 1. In synapses on neuronal perikarya

(axo-somatic) and in certain of those on dendrite trunks, on the other hand, the percentage of the length of the apposed membranes showing increase in density and thickness is small. Also there is no marked difference between the thickening of the pre- and post-synaptic membranes. These are termed type 2.

Type 1 synapses. In Pl. 1, figs. 1 and 3, type 1 synapses are shown where the increase in thickness and density (arrow) is present over 90–100 % of the contact region between pre- and post-synaptic processes. In Pl. 1, fig. 1, the post-synaptic component is a dendrite seen in transverse section and in Pl. 1, fig. 3, a dendrite in oblique section.

The contact region is shown in detail in Pl. 1, fig. 2. The synaptic vesicles lie near the pre-synaptic membrane (*a*), which shows irregular densities along its cytoplasmic surface. The post-synaptic membrane (*c*) is situated about 300 Å. away. Material associated with its cytoplasmic surface makes the post-synaptic membrane appear thicker (up to 400 Å.) and often denser than the pre-synaptic membrane (see also Pl. 1, figs. 1, 3 and 4). In the clear zone between the pre- and post-synaptic membranes and strictly confined between their thickened regions, is situated a band of material (*b*, Pl. 1, fig. 2), showing variations in density along its length. This intermediate band is sometimes situated asymmetrically nearer the post- than the pre-synaptic membrane (Pl. 1, figs. 2, 3; Pl. 4, figs. 14, 15); otherwise it appears equidistant between the two, but never nearer the pre-synaptic membrane.

At the thickened regions the pre- and post-synaptic membranes lie parallel and about 300 Å. apart, whereas in general in the cerebral cortex the cell processes lie only about 200 Å. apart (Pl. 1, figs. 1*a*, 3*a*, 4*a*; Pl. 4, fig. 13, *a*), although the distance increases where three processes meet or occasionally elsewhere, especially near the damaged edge of the slice of cortex (see also §5).

Where the pre- and post-synaptic membranes are not thickened along their entire length, the difference in spacing is especially clear (Pl. 4, figs. 14, 15). On the right the apposed membranes are unthickened and they lie about 200 Å. apart. Where the thickening commences the membranes move apart (arrows) so that the gap increases to about 300 Å. and contains the intermediate band. Occasionally the apposed synaptic membranes are thickened in two regions, in which case the gap in the region between closes to about 200 Å. and the intermediate band disappears.

As mentioned above, the percentage length of thickening of the apposed synaptic membranes is large. Measurements on 75 type 1 synapses showed that in 88 % the thickened regions occupied 70–100 % of the total length of apposition. The mean of the absolute length of thickening seen in sections was 0.46 μ . The longest thickened region so far observed measured 1.1 μ .

Type 2 synapses. Observations of a large number of perikaryal surfaces have shown that axo-somatic contacts are present, but since they appear so consistently different in certain respects from those described above, they have been classified as a second type. As in type 1, the pre-synaptic process sometimes contains mitochondria (Pl. 2, fig. 8). However, the thickened regions (arrows) occur over only a small proportion of the length of apposition between pre-synaptic and perikaryal membranes (see below), and at these points the distance between the membranes often shows no obvious increase; nor does the intermediate band appear clearly defined. Also, unlike type 1 synapses, the thickening associated with the post-synaptic

membrane is not obviously wider than that associated with the pre-synaptic membrane (compare Pl. 2, fig. 8, with Pl. 1, figs. 1–4). One apparent exception has been observed, however, where the membranes showed an obvious increase in distance apart at the thickened regions in an axo-somatic contact (Pl. 2, fig. 9, *a*) and the intermediate band (*b*) could be clearly observed, in this case situated very close to the post-synaptic (perikaryal) membrane. In other respects this contact could be classified as a type 2 synapse.

These features make type 2 synapses inconspicuous, especially at low magnifications. Pl. 3, fig. 11, shows a type 2 synapse (circle) with thickened membranes (*a*) and vesicles in the pre-synaptic process. Another type 2 synapse is shown on the base of the apical dendrite of the same neuron (Pl. 3, fig. 10, *a*) and the pre-synaptic process makes a second type 2 contact (*b*) on a small dendrite (*c*) (seen in cross-section, with tubules scarcely visible). Compare a type 1 synapse (circle, Pl. 3, fig. 10) with these type 2 synapses.

Type 2 synapses also occur on the trunks of the smaller dendrites (Pl. 2, fig. 5). Here a single thickened region is shown but more than one thickened region along the synaptic membranes can sometimes be seen.

As mentioned above, the percentage length of thickening of the apposed membranes in type 2 synapses is small. Measurements on 47 axo-somatic (type 2) synapses showed that the percentage length of thickening in 90 % of the cases did not exceed 40 % (compare type 1). The mean for absolute length in sections was $0.25\ \mu$. The longest length of thickening so far observed measured $0.45\ \mu$.

Clear processes (Pl. 3, figs. 10, 11, *d*) also make contacts with the neuron surface as described by other workers. Some can be identified as neuroglial since they can sometimes be traced to their cell-bodies. Other contacting processes (*e*) can be identified as axon sections, because of the clusters of characteristic vesicles (200–600 Å. in diameter). In these cases the plane of section might not have passed through the thickened regions of the membranes.

Other processes directly related to the neuron surface include myelinated axons, neuroglial cell-bodies, dendrites, cell-bodies of other neurons and possibly the basement membranes of blood vessels. These relationships will be considered in detail elsewhere.

5. THE CEREBRAL CORTEX FIXED WITH POTASSIUM PERMANGANATE

The uses and limitations of KMnO_4 have been described by Luft (1956). Its use for study of the cerebral cortex is limited in several respects. The membranes appear clearer than with osmium fixation, but in both types of preparation apparent discontinuities appear in the membranes. This seems due to oblique sectioning (Williams & Kallman, 1955) rather than poor preservation or 'staining', and is especially obvious in sections of grey matter, where the cell processes frequently change direction of orientation. Also if the lack of large extracellular spaces seen in osmium-fixed cortex is considered a true representation, then the spaces (Pl. 2, fig. 6, *a*) in KMnO_4 -fixed material must be considered artefacts. Also after this method of fixation neural and glial processes can seldom be distinguished, for perikaryal inclusions (in particular the granules of the endoplasmic reticulum) and dendrite tubules are invisible. These tubules have been termed endoplasmic reticulum by

Palay (1956), although they do not appear in KMnO_4 preparations, whereas the endoplasmic reticulum of the cell-body does.

Pre-synaptic processes can be identified by their vesicles (Pl. 2, fig. 6). The apposed membranes (*b*) show little if any specialized regions of increased density in contrast to osmium preparations. Nor is a discrete intermediate band apparent in the synaptic cleft, although a vague density is sometimes seen.

The use of permanganate is important in the study of membranes at high magnifications (see Robertson, 1959), especially in the study of the 75 Å. unit membrane structure, which Robertson has shown to be a feature of cell membranes in general. Their presence is confirmed in cell processes of the cerebral cortex (Gray, 1959*b*). The contact regions are shown in Pl. 2, figs. 6, 7. Each membrane (*a*) has the unit configuration of two 20 Å. dense lines with a 35 Å. clear zone between. As in osmium preparations, the synaptic cleft is wider (250–300 Å.) than between the more closely apposed membranes (i.e. where shrinkage is presumed to be least) of non-synaptic regions (e.g. Pl. 2, fig. 6, *c*).

In osmium-fixed peripheral nerves, when the membranes between processes are closed in certain situations, a third dense line is observed between them. This complex has been termed an external compound membrane (Robertson, 1959). However, the over-all dimensions eliminate the possibility that the intermediate band, seen between the thickened synaptic membranes in osmium preparations, is the middle line of an external compound membrane. The intermediate band can, in fact, be identified as extracellular in position.

6. SYNAPTIC CONTACTS ON DENDRITE SPINES

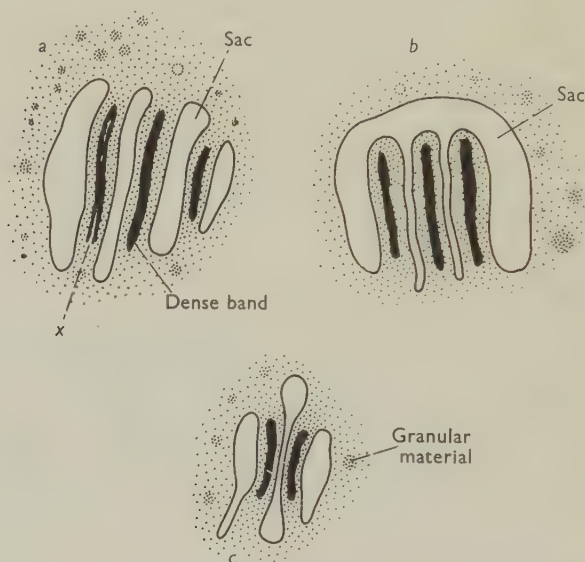
When stained by the Golgi or methylene-blue method for light microscopy, dendrites of the cerebral cortex and elsewhere are seen to have numerous spinous projections, whose function has been much disputed (see, for example, Cajal, 1911, 1954; Sholl, 1956; Fox & Bernard, 1957; Gray, 1959*b*). Here dendrite projections are described, which are seen to be sites of synaptic contact and are thought to correspond with the dendrite spines of light microscopy (Gray, 1959*b*).

Dendrite spines may be long (up to $2\ \mu$) (Pl. 4, fig. 12), or short stumps (Pl. 4, fig. 16). At the distal end of the spine, which can be either flattened or rounded, a pre-synaptic process makes contact. This has always been identified as a type 1 synapse so far. The spine cytoplasm often contains a structure that does not occur in the dendrite trunk or perikaryon. This is termed a spine apparatus.

The *spine apparatus* (Pl. 4, figs. 12, 13 and 16; Text-fig. 1) consists of two, three or more membrane-bound spaces, here referred to as sacs, although they might represent continuous channels. The sacs lie 300–500 Å. apart and only occasionally have they been seen to be connected (Text-fig. 1, *b*). Between each sac a dense band, 150–200 Å. wide, occurs, which occasionally appears as a double structure (*x*). This zone containing the band has dense cytoplasm and the zone inside the sacs appears clear in contrast. The spine cytoplasm surrounding the apparatus often contains discrete or clumped granules (diameter ~ 100 Å.) and occasional ring profiles. Clear evidence that the dendrite tubules enter the spine has not yet been attained.

Often 'isolated' pairs of pre- and post-synaptic processes appear in sections of

the neuropil. In this case, when the post-synaptic process contains the apparatus (Pl. 4, fig. 13), it is presumed to be an oblique section through the distal region of a spine. Often part of the stalk of the spine is also seen, and, of course, in a small proportion of cases continuity with a dendrite is observed.



Text-fig. 1. Diagrams to illustrate the various arrangements seen in the spine apparatus of dendrites of the cerebral cortex. Sacs alternate with dense bands. The bands sometimes appear as double structures (*x*). The sacs may be continuous (*b*) or very narrow in certain regions (*c*). Granular material is also present in the spine cytoplasm.

7. A NOTE ON AXONS AND THEIR PRE-TERMINAL REGIONS

Certain perikaryal processes, thought to be cones of origin (to be described in detail elsewhere) are occasionally encountered in sections. They contain bundles of dense tubules, which run a remarkably straight course into the axon. The axon membrane appears straight and not indented by various contacting processes, in contrast to the dendrites. So far axons have only been followed a few microns into the initial segment, and the beginning of a myelin sheath has not been encountered.

Myelinated axons are, of course, common in the grey matter forming the cerebral cortex. Very occasionally a section shows the region of terminal myelin and the unmyelinated axon can be seen continuing to form a pre-synaptic process. This contains synaptic vesicles, which are aggregated in the region of the pre-synaptic membrane.

In Fig. 24 a node of Ranvier of the cerebral cortex is shown to illustrate the appearance of myelin, where it ends (at *x* and *y*). Pl. 6, fig. 20 shows an axon losing its myelin just as at the node. The unmyelinated axon expands and makes a synaptic contact at *x*. The post-synaptic process is identified as a section of a dendrite spine since it contains a spine apparatus (Pl. 6, fig. 21—continuation of the same region, serial section). The distance from terminal myelin to synaptic contact

is in this case $1.5\ \mu$. Serial sections showed that the same process made another synaptic contact on what is presumed to be another dendrite spine (Pl. 6, figs. 20, 22, *y*) in this case only $0.5\ \mu$ from the myelin termination. In Pl. 6, fig. 23, a bouton is shown making a synaptic contact (type 2) on a dendrite immediately after the termination of the myelin.

On the other hand, unmyelinated axons can be followed for 4 or $5\ \mu$ before synapsing. An unmyelinated axon is shown in Pl. 5, fig. 19, running for $3\ \mu$, containing a few tubules, and forming a bouton on a dendrite (seen in cross-section).

Such unmyelinated axons do not exceed $0.5\ \mu$ in diameter in longitudinal sections. Serial longitudinal or transverse sections are, of course, needed to determine their true diameters, but since longitudinal sections should occasionally pass through the widest regions, the diameters of unmyelinated axons are not likely to exceed this figure. Boutons usually appear rounded in profile and seldom exceed $1.5\ \mu$ in diameter in agreement with the figures given by Armstrong & Young (1957) for light-microscope preparations.

DISCUSSION

The initial problems are:

- (1) What are synaptic contacts?
- (2) Are they all alike in thin sections: (*a*) in different positions; (*b*) regardless of function, either at the moment of fixation or in the more remote past?

To answer these questions criteria for the identification of neuronal cell-bodies, the axons and their boutons, dendrites, and neuroglia seen in thin sections are required. Mainly satisfactory ones are available for the cell-bodies and axons. Some dendrites are readily identified by their tubules and other features and certain spinous processes can be seen attached to undoubted dendrites. Other fibres are seen that are probably dendrites but cannot rigidly be proved to be such. Boutons are identified by their vesicles. Criteria exist for some but not all glial processes (see Luse, 1956; Farquhar & Hartmann 1957; Schultz *et al.* 1957; Gray, 1959*c*).

Using these criteria we can be certain that the thickened membranes occur at synapses, because a process (containing vesicles) can be seen connected with a myelin-bearing axon and making contact with a tube-carrying dendrite or its spine (Pl. 6, figs. 20–23). When two apposed processes show the thickenings the clusters of vesicles are always present in one of the processes (the pre-synaptic one).

One cannot be certain, of course, that such a situation always represents a synapse, although this is considered probable. Doubts would arise if either the presumed pre- or post-synaptic process could be shown to be neuroglial. In the cortex undoubted neuroglial processes (seen in the section to be in continuity with their cell-bodies) have never been seen to have the thickened membrane-vesicle feature and, when the evidence of other workers (Introduction) is taken into account, there is little doubt that this feature is specific to the pre-synaptic processes of axons (see below).

Nor is there any evidence to indicate that the presumed post-synaptic component can be glial and not neuronal. Frequently the post-synaptic process appears clear or faintly granular and unless its origin is determined by serial sections, it cannot be decided whether it is a section of a dendrite trunk, spine or neuroglial process. Dendrites can sometimes appear clear if cut obliquely or longitudinally for in these situations the dendrite tubules lose contrast (§3) and may become invisible. This

effect is especially evident when phosphotungstic acid is omitted from the technique. A section through a dendrite spine, which misses the spine apparatus, is another situation where the post-synaptic process can appear clear.

Schultz *et al.*, (1957) have also observed 'synaptic' contacts on clear processes. However, these authors assumed that clear processes were specifically neuroglial and doubted that the features of the contacts (i.e. 'pre-synaptic' vesicles associated with thickened membranes) were valid criteria for identifying synapses. Since dendrites or their spines can appear clear in section, their doubts are not justified.

The author has found no evidence to show that neuroglia have synaptic contacts. Neuroglial cell-bodies and processes that can clearly be seen to originate from them, also the large clear end-feet on blood vessels, which are for several reasons likely to be glial processes (Luse, 1956; Schultz *et al.* 1957; Gray, 1959*c*) have been carefully examined and have not yet been found to occur as a post-synaptic component in a synapse.

Conversely, one cannot be certain that all synapses carry thickened membranes with associated pre-synaptic vesicles. Processes with vesicles but no thickened membrane regions are commonly observed. It could be argued (*a*) that the thickening is not in the plane of section, but would be observed in serial sections of the pre-synaptic process, or (*b*) that the thickenings are not permanent structures, but are produced during synaptic activity and then disappear. A study of the effects of degeneration of the pre-synaptic process would show whether or not the post-synaptic thickening is permanent. (*c*) They may be temporary structures, but when present, not related to synaptic activity.

In addition, there is the possibility that inter-neuronal contacts which show no specializations of their apposed membranes, may be sites of transfer of information. Occasionally a pair of boutons or dendrites, or a dendrite and a nerve cell-body, have membranes in apposition (200 Å. apart). It seems probable that there would be electrical interaction between these processes and if this can be shown to be more than chance interference, then such contacts can justifiably be termed synaptic.

Palay (1956) and others have suggested that the thickened regions might be the actual transmission points and/or adhesion regions analogous to the terminal bars of epidermal cells. Direct evidence from material damaged in preparation is given in this paper to support the latter view. If the situation in the central nervous system requires that synaptic contacts, once made, should remain fixed, then it is logical to suppose that adhesive and transmitting regions coincide.

The type 1 synapses described here show several morphological similarities to neuro-muscular junctions (Reger, 1957; Robertson, 1956, 1959). Pre-synaptic vesicles, of course, occur in both situations (Palay, 1956). In the synaptic gap a dense band is situated, which is perhaps homologous with the intermediate substance situated between the membranes of the neuro-muscular junction. There also, just as in the synapse, the post-synaptic membrane shows a greater thickening and density than the presynaptic membrane. Finally in both cases the apposed membranes are more widely separated than in non-synaptic regions. In both cases the intermediate layer may be cementing in function or perhaps also more directly concerned with the transmission mechanism. It is relevant that a band of extra-cellular material occurs between the apposed thickened membranes of the contact

zones of epidermal cells; here also the membranes are more widely separated (see Odland, 1958). However, in epidermal contacts, the two thickenings are of equal dimensions, the intermediate line is situated symmetrically in the centre of the cleft, tonofibrils are present and vesicles absent.

The occurrence of material producing a greater thickening in the post- than in the pre-synaptic membrane is not accounted for at present. This effect is still seen, although less clearly, when phosphotungstic acid is omitted from the technique (Methods (b)). The marked post-synaptic thickening described here in type 1 synapses is not a feature of synaptic contacts that have been described in other regions of the mammalian central nervous system (see Palay, 1956, 1958). The effect might be attributed to the use of phosphotungstic acid. However, in this case, one would expect the effect to be observed in all the post-synaptic thickenings, whereas in fact the post-synaptic thickening of axo-somatic and certain axo-dendritic synapses differs little from the pre-synaptic one. This is one of the reasons for considering axo-somatic contacts in a separate category (type 2). In fact it seems that the type 2 synapses correspond with those described by Palay (1956, 1958) in other regions of the central nervous system. They show, in common with type 2 and in addition to the feature just described, the occurrence of thickenings only over short distances of the apposed membranes, and only vague and irregular densities in the synaptic cleft (see Palay, 1956) and no clearly defined intermediate band. Perhaps type 1 synapses are a special feature of the cerebrum. However, other regions of the central nervous system must be examined by the phosphotungstic acid method before this conclusion can be reached.

At present there is no evidence to suggest that type 1 and type 2 synapses are functionally different. Even the morphological (i.e. three-dimensional) reality of these structures seen in thin sections must be carefully considered. For example it can be argued that the thickenings of the contact regions are oval or circular apposed plates (see Sjöstrand, 1956) and that around the margins the thickening of the post-synaptic plate decreases, the intermediate band becomes less defined and the membranes begin to close. Thus a section through the margin would produce a type 2 synapse and one through the centre a type 1 synapse. However, if this were so, then one would expect at least a proportion of axo-somatic synapses to appear in the type 1 category and a proportion of contacts on dendrite spines to appear in the type 2 category, but this has not been observed. Clearly serial sections are needed to decide this issue.

One wonders why so many of the synaptic contracts should be poised on spines away from the dendrite trunk (Gray, 1959*b*). Golgi preparations of the visual cortex show dendrites commonly covered with spinous processes. It is important to remember that in Golgi preparations only about one in seventy neurons with their dendrite ramifications is visible (Sholl, unpublished). The cortex is in fact packed with dendrites and their spines. At present it cannot be stated that all spines are sites of synaptic contact or that they all contain a spine apparatus. These features represent a new and intriguing situation for physiological investigation.

SUMMARY

1. The method found most suitable for the study of the cerebral cortex included fixation with osmium tetroxide, 'staining' with phosphotungstic acid and embedding in araldite.

2. Frequently, apposed membranes of two processes show thickened regions and clusters of vesicles near the thickened membrane of one of the processes. These are thought to be synaptic contacts, because:

(a) The vesicle-bearing (pre-synaptic) process can be seen to originate from a myelinated axon.

(b) The other (post-synaptic) process can be identified as a nerve cell-body, or a dendrite trunk or a dendrite spine.

(c) No such contacts have been observed on neuroglial cell-bodies or their processes.

(d) The thickened regions show special adhesive properties.

(e) Of the similarity with neuro-muscular junctions and correspondence with descriptions by other workers of synaptic contacts in other regions of the vertebrate nervous system.

3. Two categories of synaptic contacts are distinguished: (a) type 1 synapses, which occur on dendrite trunks and dendrite spines; (b) type 2 synapses, which occur on dendrite trunks and neuron cell-bodies.

4. In type 1 synapses a large percentage of the length of the apposed membranes shows increased thickness and density. The post-synaptic thickening is more pronounced than the pre-synaptic thickening. These thickened regions of the membranes lie farther apart than where the apposed membranes are unthickened and in the extracellular region between the thickened membranes an intermediate band of material can be seen.

5. In type 2 synapses the percentage length of thickening is small, the pre- and post-synaptic thickenings are of similar dimensions, the intermediate band is not clearly visible and the membrane spacings at these regions differ little from the non-thickened regions.

6. Dendrite spines are sites of synaptic contact (type 1). The spine cytoplasm contains a structure termed a spine apparatus which consists of a group of 'sacs' separated by bands of dense material.

I am indebted to Prof. J. Z. Young, F.R.S., Dr J. D. Robertson, Mr. D. A. Sholl, and Mr K. C. Richardson for advice and constructive criticism: also to Mrs R. Wheeler, Mr B. Cowley and Mr A. Aldrich for technical assistance.

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KEY TO PLATES

<i>ax</i>	axon (myelinated)	<i>post</i>	post-synaptic process
<i>ci</i>	cisternae of neuron endoplasmic reticulum	<i>pre</i>	pre-synaptic process
<i>cyt</i>	neuron cell-body cytoplasm	<i>s</i>	spine apparatus
<i>den</i>	dendrite	<i>sp</i>	dendrite spine
<i>g</i>	granules of the endoplasmic reticulum	<i>sv</i>	synaptic vesicles
<i>m</i>	mitochondria	<i>t</i>	dendrite tubules
<i>my</i>	myelin sheath	<i>tu</i>	axonal tubules
<i>nuc</i>	nucleus of neuron	<i>unmy</i>	unmyelinated axon

Other letters: see text and individual captions.

EXPLANATION OF PLATES

Visual cortex of adult rats. Figures 6 and 7 are of KMnO_4 -fixed material. All others, OsO_4 -fixed, phosphotungstic acid stained.

PLATE 1

- Fig. 1. Dendrite seen in transverse section containing tubules. It has a type 1 synapse. The pre-synaptic process contains vesicles. Arrow indicates thickened membranes at contact region.
 (a) Non-thickened regions of membranes (see text).
 Fig. 2. Details of the thickened apposed membranes (*a* and *c*) of pre- and post-synaptic processes. A band of extracellular material (*b*) occurs in the synaptic cleft. The post-synaptic process is probably a dendrite spine.
 Fig. 3. A type 1 synapse on a dendrite seen in longitudinal section. See fig. 1 for lettering.
 Fig. 4. A group of synaptic contacts on a small dendrite. They are all type 1. Note how the vesicles of the pre-synaptic process are clustered near the thickened regions. The dendrite also has a spine synapse (bottom right). (*b*) Region of dendrite where tubules are not seen. (*a*) See text.

PLATE 2

- Fig. 5. A type 2 synapse on a small dendrite. Note apparent anastomoses of the dendrite tubules.
 Fig. 6. A synaptic contact in material fixed with KMnO_4 . The post-synaptic process is probably a dendrite spine. Part of the contact region is enlarged in fig. 7. (*a*) Extracellular spaces seen when this fixative is used. (*b*, *c*) See text.
 Fig. 7. Enlarged portion of fig. 6. The triple structure of the pre- and post-synaptic membranes (*a*) can be seen (KMnO_4 fixation).
 Fig. 8. Type 2 synapses on a neuron cell-body that contains the characteristic granules and cisternae of the endoplasmic reticulum. Arrows indicate thickenings of the synaptic membranes.
 Fig. 9. Contact region of a synapse on a neuron perikaryon. Here the intermediate band (*b*) can be seen in the synaptic cleft: this is uncommon in an axo-somatic contact. (*a*) Region where distance apart of membranes increases.

PLATE 3

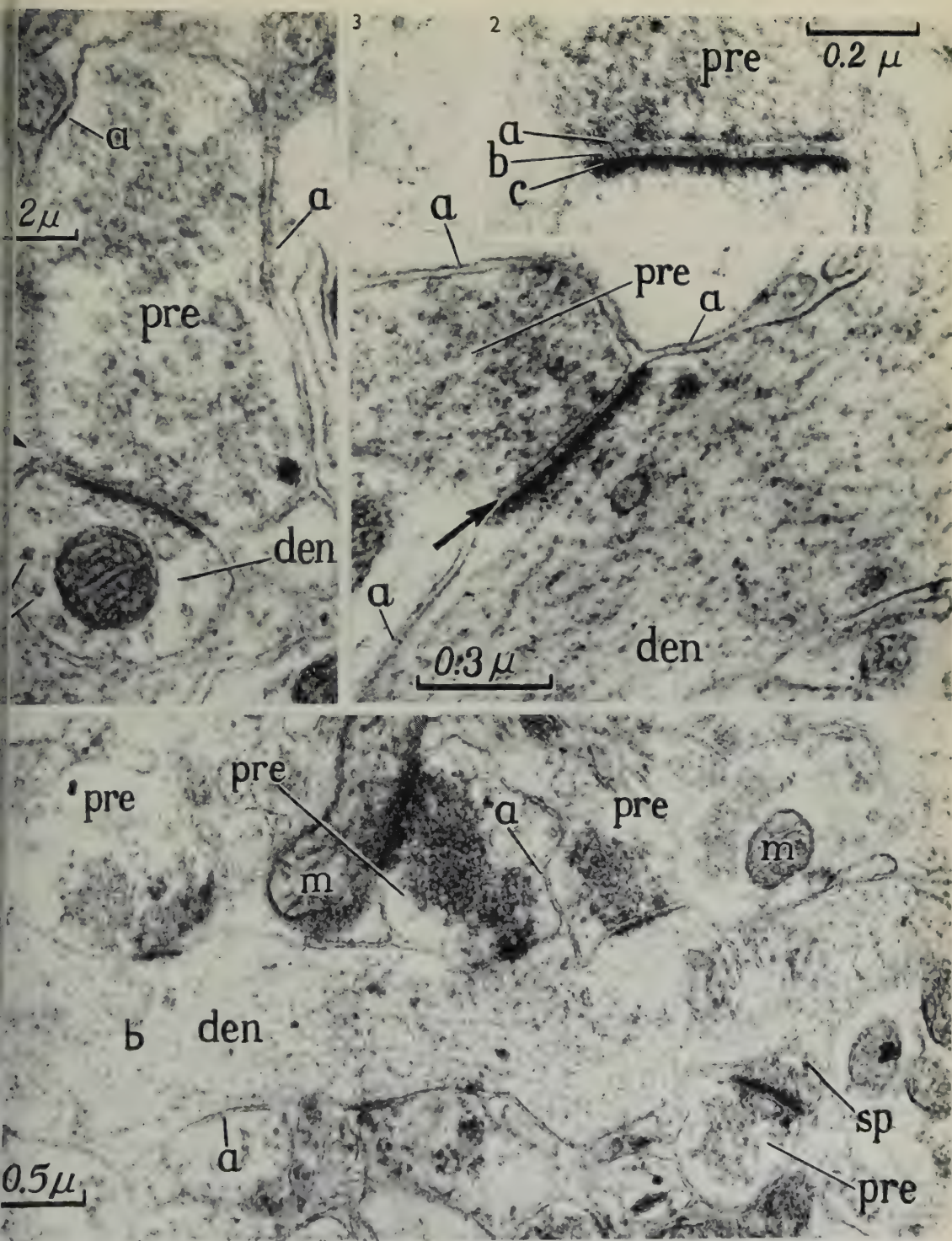
- Fig. 10. Base of an apical dendrite of a pyramidal neuron. Type 2 synapse at (*a*). Type 1 synapse (in circle) in the neuropil. (*d*) Presumed neuroglial process. (*e*) Axon terminals. (*b*, *c*) See text.
 Fig. 11. Type 2 synapse (in circle) on neuron cell body. (*a*) Thickened region of synaptic membranes. (*d*) Presumed neuroglial process.

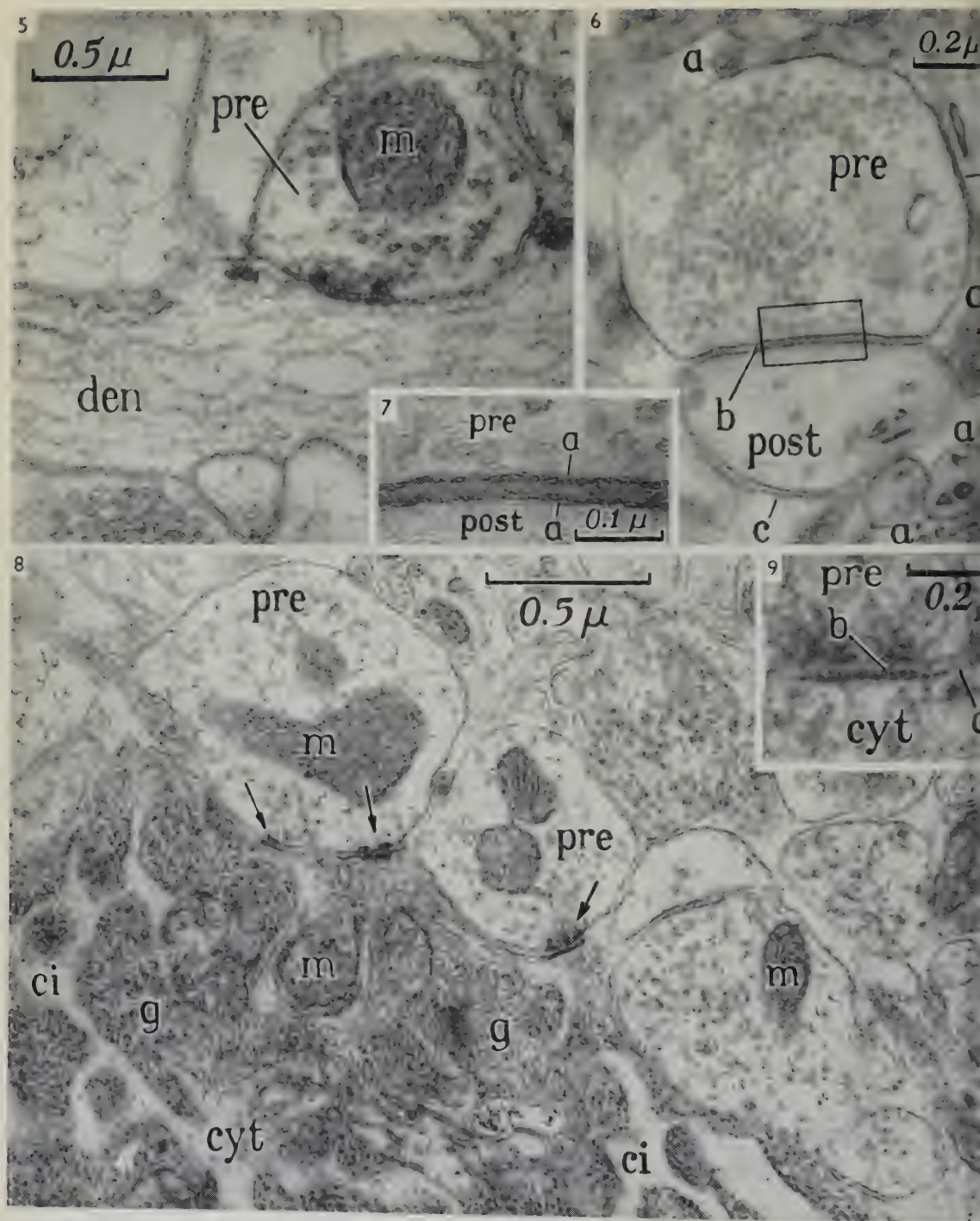
PLATE 4

- Fig. 12. Dendrite with spine. The spine contains a spine apparatus and has a type 1 synapse at its apex.
 Fig. 13. Type 1 synapse. The isolated section of the post-synaptic process is identified as a dendrite spine profile since it contains a spine apparatus. (*a*) See text.
 Figs. 14, 15. Type 1 synapses. The membranes of the apposed synaptic processes become wider (arrow) at the thickened regions and the intermediate band is seen in the cleft between the thickened regions.
 Fig. 16. Dendrite with two short spines each with a spine apparatus and a type 1 synaptic contact.

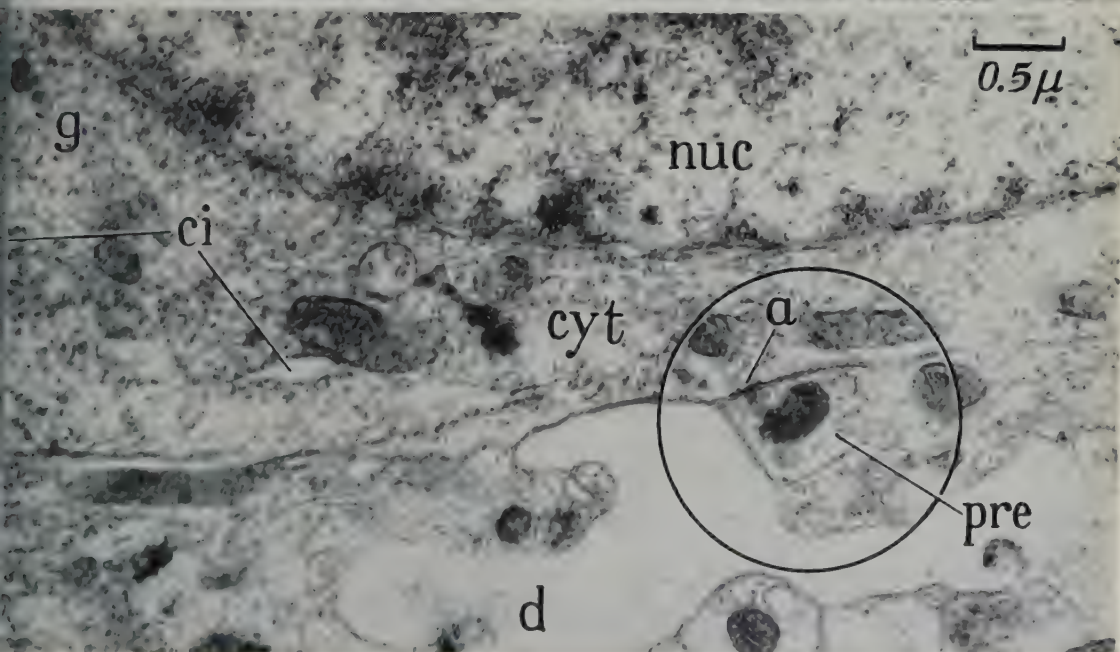
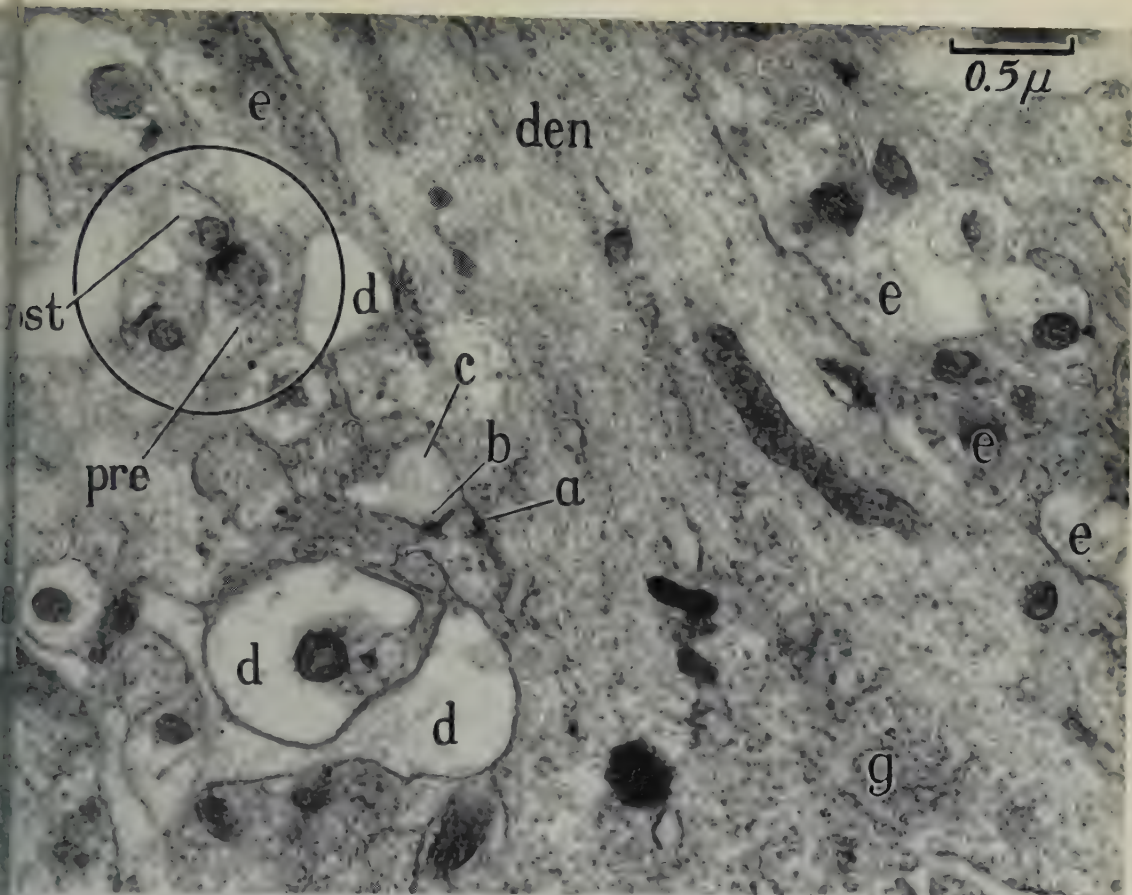
PLATE 5

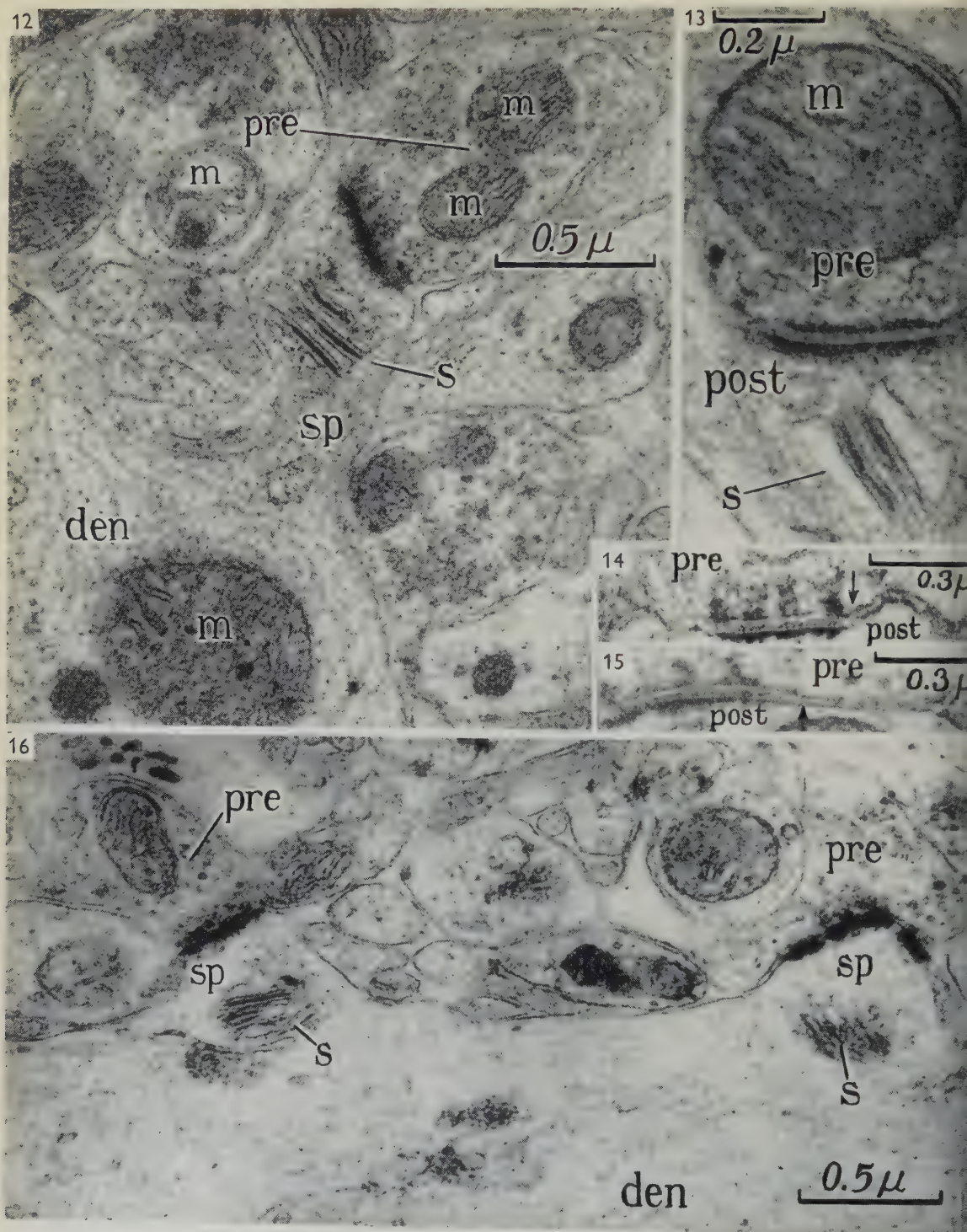
- Fig. 17. Large dendrite with tubules orientated (*a*) normally, (*b*) obliquely, and (*c*) longitudinally to the plane of section. Different directions of orientation are related to branching or change of direction of the dendrite.
 Fig. 18. Ruptured processes appear at the margins of cortical slices, where damage is caused during preparation. The thickened regions of the synaptic membranes remain firmly attached.
 Fig. 19. Unmyelinated axon containing a few tubules in its pre-terminal region. It forms a bouton on a small dendrite containing the characteristic tubules (seen in cross-section).



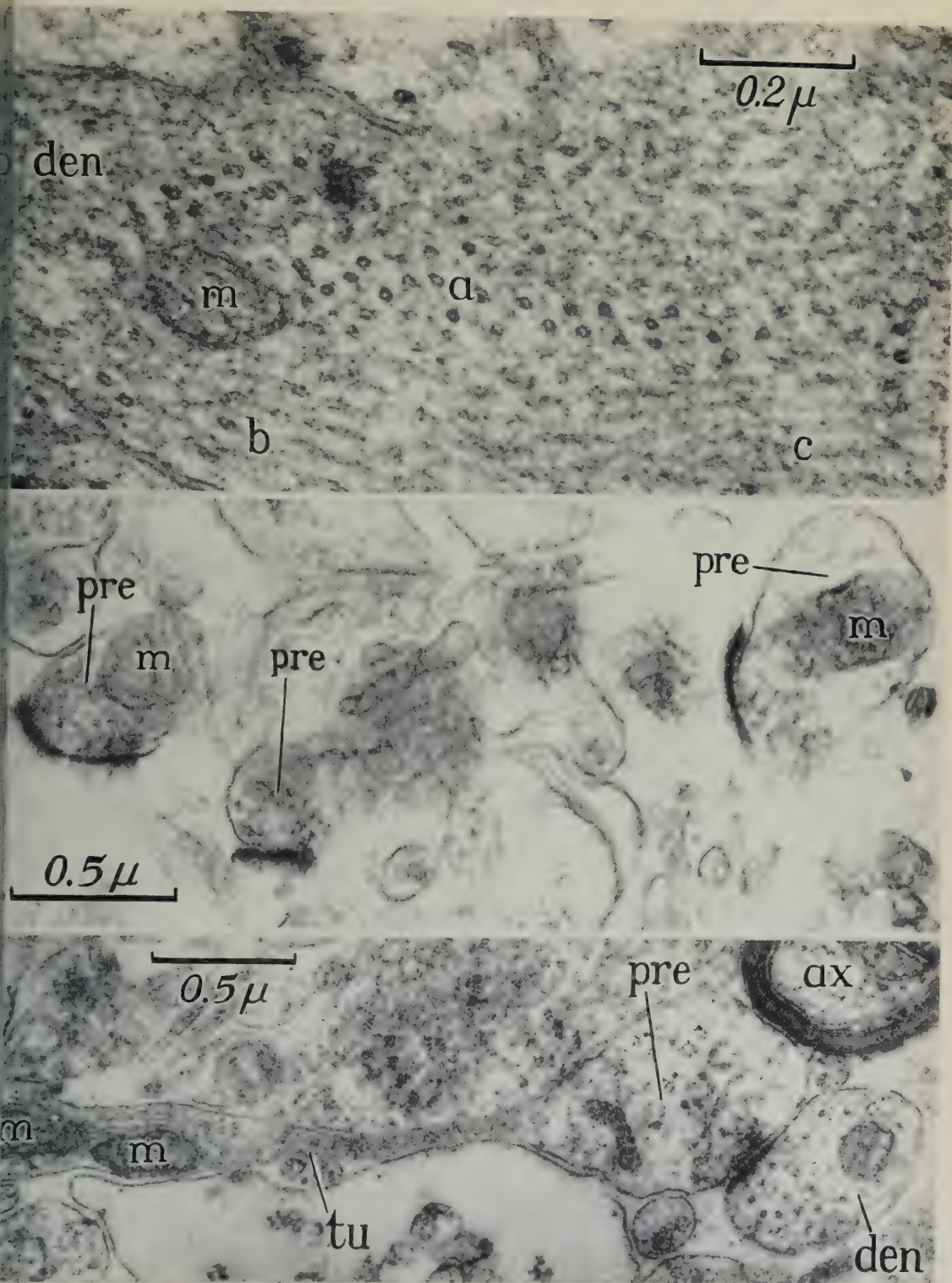


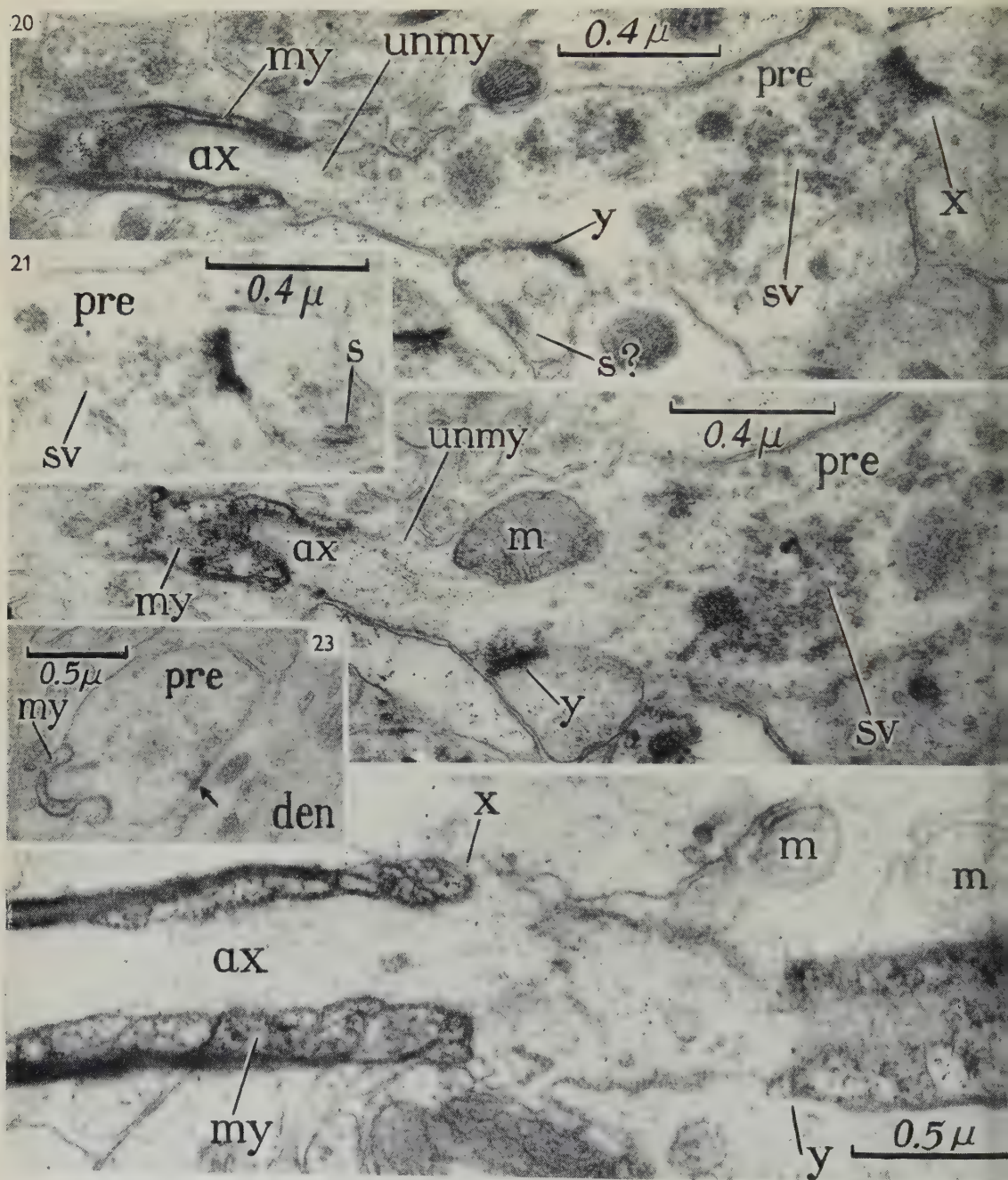
GRAY—AXO-SOMATIC AND AXO-DENDRITIC SYNAPSES OF CEREBRAL CORTEX





GRAY - AXO-SOMATIC AND AXO-DENDRITIC SYNAPSES OF CEREBRAL CORTEX





GRAY—AXO-SOMATIC AND AXO-DENDRITIC SYNAPSES OF CEREBRAL CORTEX

PLATE 6

- Fig. 20. A pre-synaptic process seen originating from a myelinated axon. The process contains synaptic vesicles and makes synaptic contact at *x* with a dendrite spine—see figs. 21 and 22.
- Fig. 21. Serial section of fig. 20. The post-synaptic process (of fig. 20) is shown to be a dendrite spine, since it contains a spine apparatus (*s*).
- Fig. 22. Serial section of fig. 20. The pre-synaptic process makes a second synapse at *y*. The second post-synaptic process is also thought to be a dendrite spine, because of its shape and because it contains a vague structure (*s*?, fig. 20), which, however, cannot clearly be identified as a spine apparatus.
- Fig. 23. A type 2 synapse on a dendrite trunk. The pre-synaptic process is seen emerging from a terminating myelin sheath. Arrow shows thickened contact region.
- Fig. 24. A node of Ranvier of the cerebral cortex, included so that the terminating myelin configuration (*x, y*) can be compared with figs. 20, 22 and 23.

A NOTE ON THE NEURONAL PACKING DENSITY IN THE CEREBRAL CORTEX

By D. A. SHOLL

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Owing to a misinterpretation of Dr Haddara's data, the figures for the mouse visual cortex published in the paper 'A comparative study of the neuronal packing density in the cerebral cortex' (Sholl, 1959; pp. 143-158) are incorrect.

The values printed in Table 2 should be amended so that the mean number of neurons in a cortical cylinder with cross-sectional area $400 \mu^2$ in the mouse visual cortex is 48 with a range of 46-51. The corrected values of neuronal densities for this cortex (Table 3) are as follows:

General Summary of neuronal densities at increasing depths of the mouse visual cortex

Type of cortex	No. of counts	Mean packing density of perikarya/ $10^6 \mu^3$ of cortex in terms of relative depth with corresponding standard deviation of mean.									
		0.1	S.D.	0.2	S.D.	0.3	S.D.	0.4	S.D.	0.5	S.D.
Mouse visual (MV)	5	258.0	9.9	245.4	2.1	232.8	9.9	235.8	12.3	157.8	10.5
		0.6	S.D.	0.7	S.D.	0.8	S.D.	0.9	S.D.	1.0	S.D.
Mouse visual (MV)	5	145.2	6.3	195.6	7.2	181.8	11.7	183.6	3.9	117.6	17.4

Figure 7 should be replaced by Figure 1 of this note.

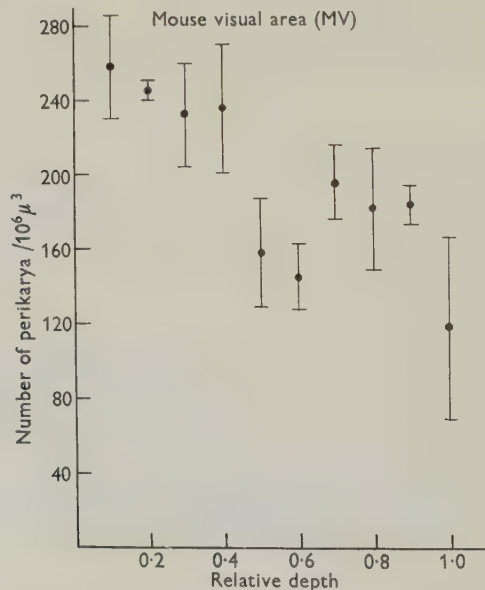


Fig. 1. Mean values of the neuronal densities at different cortical depths with 95 % confidence limits for mouse visual cortex.

In Figure 9 the graph for the mouse (MV) remains the same shape but should be moved upwards, the new graph being parallel to that illustrated.

These amended figures show that the neuronal packing density in the mouse visual cortex was systematically underestimated in the original paper. The corrected values will increase the Generalized Distance of the mouse cortex from the other cortices studied making this cortex even more different from those cortices. The general conclusions of the paper remain unaffected.

REFERENCE

- SHOLL, D. A. (1959). A comparative study of the neuronal packing in the cerebral cortex. *J. Anat., Lond.*, **93**, 143-158.

THE EFFECT OF TEMPERATURE ON THE MATURATION OF REGENERATING PERIPHERAL NERVES IN THE RAT

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It has been known for many years that body temperature affects the rate of nerve-fibre degeneration in both poikilothermic animals (e.g. frog, Mönckeberg & Bethe, 1899) and in hibernating mammals (Merzbacher, 1903). Recently it has been shown that variations in temperature along the length of a nerve (the ventral caudal nerve of the rat) can cause local acceleration or retardation of the process (Gamble & Jha, 1958). The regeneration which may follow can also be affected, as Rindone (1925), Lubinska & Olekiewicz (1950) and Lubinska (1952) have shown in amphibians, and Deineka (1908) and Gamble (1957, 1958) in the rabbit and rat respectively.

Regeneration is a complex process consisting of an early stage of axonal outgrowth and a later stage of maturation characterized by an increase in the calibre of the axon, and, in the case of myelinated fibres, the acquisition and growth of a myelin sheath. The cell-body of the neuron, the Schwann cells, the endoneurial tubes and peripheral connexions with appropriate end-organs are all involved, and any of them could be affected by heat with effects on the rate of outgrowth, maturation or both.

In all investigations of this problem, attempts have been made to change the temperature of the regenerating neuron or its processes by changing the environmental temperature. With poikilothermic animals like amphibians, raising the environmental temperature raises the whole body temperature and therefore warms not only the peripheral regenerating fibre but also the cell-body from which it is growing. In fully homiothermic mammals variations in environmental temperature, which are small enough to be compatible with life, have little or no effect on the temperature of deeply situated structures like the cell-body, and have a direct effect only on the peripheral part of a nerve in a limb or tail. Deineka studied regeneration in the cut sciatic nerve of a rabbit, and could have produced only slight local temperature differences with the range of environmental temperatures employed (12–14° C. and 30° C.) since the nerve is fairly deeply situated in the muscles of the thigh. He found, however, that the degree of neurotization of the peripheral stump reached in 12 days in animals kept at 12–14° C. was attained in 5 days in animals kept at 30° C. If, as he assumed, this was a direct effect of the temperature difference, it must have been caused peripherally and not by any change in the temperature of the cell-body.

Gamble (1957, 1958) studied the effects of environmental temperature on the ventral caudal nerve in the rat, and by keeping one set of animals in a cold room produced subcutaneous temperatures in the tail about 10–15° C. below those in animals at room temperature. In other animals kept in heated cages at about 35° C. the subcutaneous temperatures in the tail were about 10–15° C. higher than at room temperature. The temperature in the peritoneal cavity and presumably of the spinal

* On study leave from Darbhanga Medical College, granted by the Government of Bihar, India.

cord and dorsal root ganglia remained constant at 37–38° C. in all animals in each of the three environments. He found that the outgrowth of axons in the 'cold' animals was almost completely inhibited up to 35 days after crushing the nerve, and that in the 'hot' animals, maturation of regenerating fibres, as judged by the appearance of a myelin sheath and nodes of Ranvier, began about 10 days earlier than in animals at room temperature. It followed therefore that both the outgrowth and maturation stages of regeneration were influenced by environmental temperatures, which could have been directly effective only in the peripheral course of the nerve.

In Gamble's experiments the peripheral temperature differences were large, and the nerves were examined in teased preparations in which only comparatively small samples of the total number of fibres in the nerve could be studied. It was possible also, both in his and in Deineka's experiments, that the effect observed was not due directly to the peripheral temperature, but indirectly to a change in the metabolism of the whole animal, a 'stress' reaction as it were, which could be effective centrally as well as peripherally. It was decided therefore to study the rate of regeneration in the subcutaneous part of the course of the rat's sural nerve in different environmental temperatures. The temperature differences which could be produced in such a situation would be smaller than in the tail, but probably larger than in the sciatic nerve. The study has been limited to the stage of remyelination, where it is easy to obtain precise quantitative data for the whole nerve. The question whether effects were due directly to the local temperature, or indirectly to a generalized stress reaction, has been investigated by comparing the results obtained in the sural nerve with a similar series of experiments on the genito-femoral nerve. The latter runs for the greater part of its course on the dorsal aspect of the peritoneal cavity where its temperature is unaffected by the different environmental temperatures used.

MATERIALS AND METHODS

Young adult albino rats were used of weights ranging from 180 to 200 g. One larger animal (310 g.) was included in the series. Both male and female animals were used for the sural nerve experiments; for the genito-femoral nerve experiments all the animals were female.

Under ether anaesthesia and with full aseptic precautions an incision was made in the leg 2.5 cm. proximal to the lateral malleolus and the nerve exposed and crushed with smooth-pointed watchmaker's forceps for 10 sec. The level of the crush corresponded approximately to the level at which the nerve emerged from under the biceps femoris muscle and became subcutaneous. After dusting with penicillin and sulphathiazole powder the wound was closed. The animals were then placed in cold, warm or hot environments with cage temperatures of 3–8°, 20–22° and 34–37° C. respectively, and allowed to survive for periods of 30, 50, 70, 100 and 150 days. Intraperitoneal and subcutaneous temperatures (leg and foot) were measured with a thermocouple inserted in a hypodermic needle, the leg subcutaneous temperature being taken from near the sural nerve.

When the animals were killed the nerve was exposed and the level of the crush identified either visually, or in the case of the longer term specimens, by measuring to a point 2.5 cm. proximal to the lateral malleolus. It was cut at this level and removed for a little over 1 cm. distally and fixed, lightly stretched on a card, in

Flemming's fluid. The card was marked to identify the proximal end. The nerve was embedded in paraffin and a level marked on the block as nearly as possible 5 mm. from its proximal end. All the sections used were made at about this level, so that all were about 5 mm. distal to the original lesion. The sections were cut transversely at 5μ and stained by the Gutmann & Sanders (1948) technique for myelin sheaths.

For counts and other measurements the methods described by Quilliam (1956) were used. Photographs ($\times 750$) were made by projecting the sections directly on bromide paper. When checked, the actual magnifications were found usually to be slightly greater, the mean value for 52 observations being 755 (s.d. 8). This variation was not large enough to have a significant effect on the measurements and comparisons to be reported. The fibres were enumerated and classified in 2μ size groups. Estimates of the total cross-sectional area of all the fibres (axons and myelin sheaths) were made by multiplying the mean area of the fibres in each size group by the number in that group and adding the products thus obtained. It should be pointed out that in all cases the counts and measurements were made on all the fibres in the nerve, thus avoiding sampling errors.

Apart from the operative technique, the observations on the genito-femoral nerve were made similarly. The nerve was approached through an incision in the ventral abdominal wall and, after retracting the viscera, was exposed on the ventral aspect of the psoas muscle. It was crushed at a point 1 cm. cranial to the bifurcation of the aorta. The animals were kept for 50 days, two in hot ($34-37^{\circ}\text{C.}$) and two in cold ($3-8^{\circ}\text{C.}$) environments, this being the period at which the effects on regeneration in the sural nerve had been found to be most marked. When the animals were killed the level of the crush was identified by measurement from the aortic bifurcation and about 1.5 cm. of the nerve distal to this point was removed and treated in the same way as the sural nerve. Sections were cut from the distal end, i.e. about 1.5 cm. from the lesion, in each specimen.

OBSERVATIONS

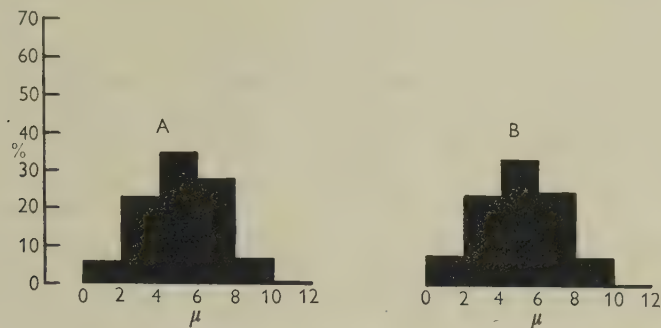
The normal sural nerve of the rat

The sural nerve of the rat runs subcutaneously in the leg, and for about 1 cm. from its emergence from under cover of the biceps muscle gives no branches, although at varying levels it becomes divided into two or three fasciculi, bound together by a common epineurium. Its characteristics were examined at two levels, the first (level A) where the nerve becomes subcutaneous and the crush lesion was made, and the second (level B) where the regenerating nerve was examined in the experimental animals.

Five normal nerves were examined at level A and four at level B; the mean number of myelinated fibres at level A was found to be 815 (s.d. 18) and at level B, 825 (s.d. 18). The histograms at the two levels (Text-fig. 1) show the characteristic unimodal distribution of a sensory nerve, with the mode between 4 and 6μ ; there is no important difference between them nor between the figures obtained for the total cross-sectional areas of the fibres ($1000\mu^2$): level A, 20.0 (s.d. 1.6); level B, 19.4 (s.d. 1.8).

The sural nerve apparently varies little from rat to rat, for in another specimen

reported by Quilliam (1956) there were 809 fibres and the histogram was very similar to those in Text-fig. 1. Apart from the fasciculation its characteristics at levels A and B are also virtually identical.



Text-fig. 1. Histograms showing the percentage distribution of fibres of different calibre in the normal sural nerve at the two levels, A and B.

The regenerating sural nerve

Forty-two regenerating sural nerves were examined, after varying periods in hot, warm, and cold environments. Pl. 1, figs. 1-3, shows the appearance of three of them after 30 days. It is obvious that the specimen kept in the cold environment (Pl. 1, fig. 3) contained comparatively few myelinated fibres and much degeneration debris; it contrasted strongly with the specimen kept in a hot environment (Pl. 1, fig. 1) where nearly all debris had been removed and many myelinated fibres were present. These were not as numerous as in the normal nerve at this level and were clearly smaller and more uniform in calibre. The specimen kept in a warm environment (Pl. 1, fig. 2) was intermediate in appearance. The condition of three similar specimens after 150 days is shown in Pl. 1, figs. 4-6. The calibre of the fibres in the 'hot' specimen (Pl. 1, fig. 4) was obviously greater than in the 'warm' specimen (Pl. 1, fig. 5); in the 'cold' specimen (Pl. 1, fig. 6) only quite fine fibres were seen. The 'warmer' specimens also contained larger total numbers of fibres, although, as will be shown below, it is doubtful if this was a significant difference.

By no means all the specimens, when examined by simple inspection, showed such an obvious contrast as those illustrated, although the differences became clear when more precise quantitative methods were employed. The results for all the specimens, so far as the total number of myelinated fibres (irrespective of their calibre) is concerned, are summarized in Table 1.

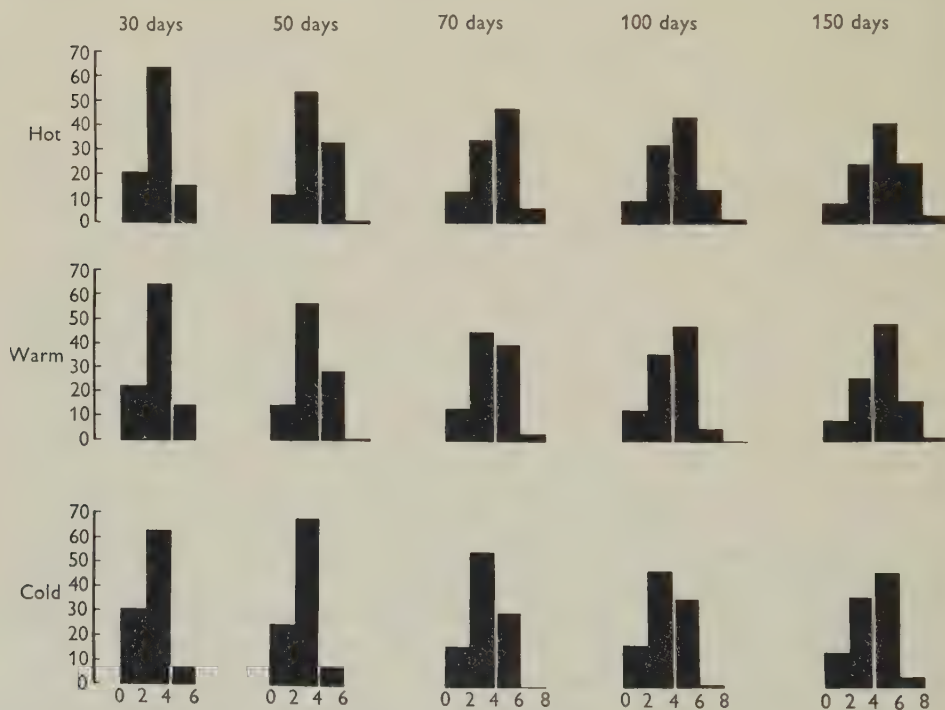
It will be seen from Table 1 that the effects of the three different environments were most marked after 50 days when the differences of the means of the hot and warm, and also of the warm and cold specimens, were both significant ($P < 0.02$). It can also be seen that by 70 days, specimens kept in a hot and a warm environment had both reached a figure not significantly different from the normal. Specimens kept for longer periods showed no further increase. The specimens kept in a cold environment appeared to lag behind throughout the period of the experiment, but it should be pointed out that at 70, 100 and 150 days the totals obtained were not

significantly different in a statistical sense from those in the warm or hot environments. It would be necessary to examine more specimens at the longer periods to establish with certainty whether the persistent lag in a cold environment was real or not.

Table 1

Days of regeneration ...	30	50	70	100	150
Hot environment (34–37° C.)	690 ± 96 (4)	868 ± 4 (2)	762 ± 47 (2)	758 ± 76 (2)	826 ± 8 (2)
Warm environment (20–22° C.)	520 ± 100 (4)	628 ± 69 (6)	782 ± 92 (2)	801 ± 90 (2)	792 ± 27 (2)
Cold environment (3–8° C.)	441 ± 83 (4)	480 ± 75 (4)	613 ± 67 (2)	681 ± 78 (2)	636 ± 76 (2)

The mean number of myelinated fibres (\pm S.D.) in the regenerating sural nerve after different time intervals in hot, warm and cold environments. The figures in parentheses give the number of specimens in each case.



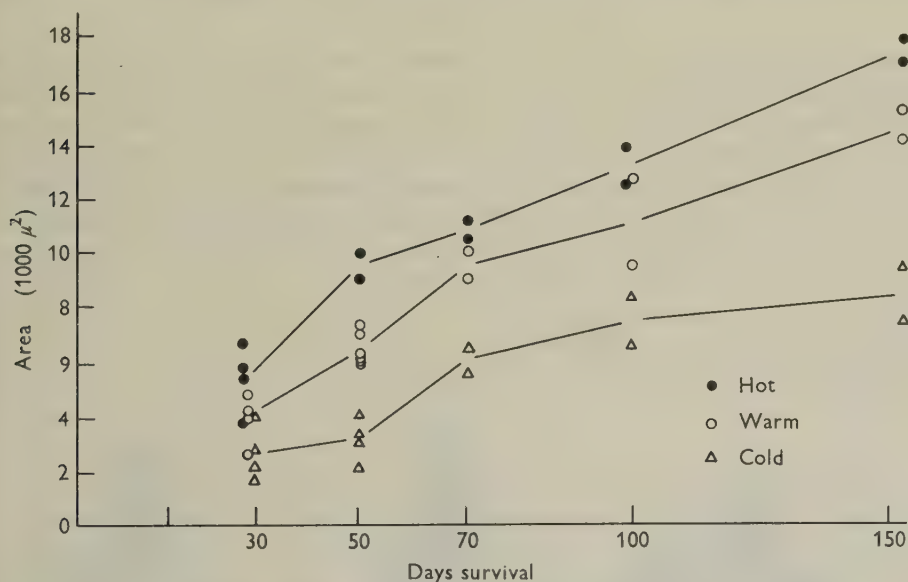
Text-fig. 2. Histograms showing the percentage distribution of fibres of different calibre in regenerating sural nerves after varying periods in hot, warm and cold environments. In each case the histogram is based on the mean values obtained from a group of specimens. The numbers of specimens in the groups are given in Table 1.

Another method of illustrating the stage of maturation reached is shown in the histograms (Text-fig. 2). Here any variation due to the numbers of fibres present has been eliminated because the different size groups are represented in each case as a percentage of the total.

If the histograms are read horizontally from left to right the expected increase in

the proportion of fibres in the larger size groups in the longer term specimens is shown by a typical 'shift to the right', in all three environments. The shift occurs more rapidly in the hot than in the warm and in the warm than in the cold environments. The normal nerve is characterized by a modal value in the $4-6\mu$ size group, and in the regenerating nerves this was reached by 70 days in the hot environment. It was not reached in a warm environment till 100 days, and in a cold environment, 150 days. The histogram at 150 days in the hot environment is very like that of the normal nerve and the situation in a warm environment is very similar. In the cold, there was still an abnormally large proportion of fibres in the $0-2$ and $2-4\mu$ categories.

If the histograms are read vertically from below upwards a similar 'shift to the right' is apparent in passing from the cold to the warm and on to the hot specimens. This shift is the effect of the environmental temperature differences, and is present at all the periods studied. By contrast, reading diagonally (downwards towards the right), very little change is seen in the histograms; for example those at 70 days (hot), 100 days (warm) and 150 days (cold) are virtually identical.



Text-fig. 3. A graphical representation of the cross-sectional area of the myelinated fibres in regenerating sural nerves after varying periods in hot, warm and cold environments. Each point represents one specimen. The lines connect the mean values for the groups of specimens in each of the three environments.

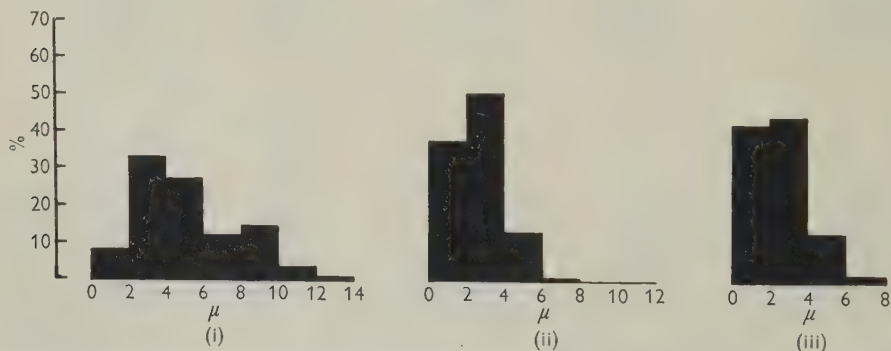
The methods of comparison so far used show differences in the total number of fibres and in the calibre distribution separately. Both total numbers and calibre are characteristic of the stage of maturation reached, and their combined effect can be estimated by taking the total cross-sectional area of the fibres in any of the specimens. The result of doing this is illustrated graphically (Text-fig. 3) where the areas for all specimens are plotted and lines have been drawn connecting the mean values for the groups of specimens in each of the three environments. Clearly the situation

is very similar to that revealed by the histograms and by simple enumeration, but there are some features which require comment.

The longer term specimens showed the lag in maturation in a cold environment quite definitely. The differences between the hot and the cold specimens at all periods are significant (P never greater than 0.05 in any case). Except at 50 days the hot-warm differences are not statistically significant. The warm-cold differences are significant at 50, 70 and 150 days ($P < 0.05$), but not at 30 or 100 days. There is no doubt therefore that maturation is slower in the cold animals than in the hot. There is some doubt, however, whether the smaller temperature difference between the hot and the warm animals has had a similar effect, although at 50 days there was a statistically significant difference between the specimens. It should be pointed out, however, that the calculated level of significance between the groups depends not only on the absolute difference of the means but to a very important extent on the number of specimens from which each mean is derived. At 70, 100 and 150 days there were never more than two specimens in any group and it is likely that larger numbers would have made it possible to demonstrate statistically significant differences.

The genito-femoral nerve

Preliminary observations on the normal genito-femoral nerves in two females and one male suggested the possibility of a sex difference both in the number and calibre of the fibres. One male nerve contained 998 fibres and two female nerves 627 and 760 fibres respectively. The histograms also differed. Both were unimodal, but those from the two female specimens were flatter and showed a greater proportion of larger fibres. For this reason only animals of one sex (female) were used in the study of the temperature effect on regeneration. The normal female genito-femoral nerve is shown in Pl. 1, fig. 7, and its histogram in Text-fig. 4 (i).



Text-fig. 4. Histograms of the genito-femoral nerve (female). (i) the normal nerve, (ii) the regenerating nerve after 50 days in a cold environment; (iii) the regenerating nerve after 50 days in a hot environment.

Four experimental specimens were studied, two being kept in a cold and two in a hot environment for 50 days after the nerve had been crushed. It was after this period that the temperature effect had been found to be most marked in the sural nerve. Pl. 1, figs. 8 and 9, shows the condition in two of these nerves, one 'cold' and the other 'hot'. There is obviously no substantial difference to be detected by simple

inspection. The regenerating fibres appear of very similar calibre. In the cold specimen they were rather more closely packed and in the hot specimen degeneration debris was rather more conspicuous. The occasional fibres of rather large calibre had perhaps escaped injury when the nerve was crushed. It is unlikely that such large regenerating fibres would be present after only 50 days. One and possibly two fibres of this kind were conspicuous in the cold specimen (Pl. 1, fig. 8).

The histograms (in each case based on the means of two specimens, Text-fig. 4 (ii) and (iii)) are also essentially similar and the total numbers of fibres do not differ significantly, the means being 720 (s.d. 65) and 746 (s.d. 115) in the hot and cold specimens respectively. The corresponding figures for the cross-sectional areas ($1000\mu^2$) are 4.7 (s.d. 0.2) and 5 (s.d. 0.3) and again do not differ significantly.

It follows that the rate of maturation of a nerve deeply situated in the peritoneal cavity was not affected by differences in the environmental temperature of the order used in these experiments. It may be noted also that the genito-femoral nerve was regenerating in a temperature of about 38°C . (that of the peritoneal cavity). The stage reached in 50 days should therefore be similar to that reached by the sural nerve in a hot environment and should be in advance of the sural nerve in a cold or warm environment.

In 50 days in a hot environment the sural nerve had attained a normal total number of myelinated fibres (Table 1) and the same can be said of the genito-femoral nerve; the sural nerve in the warm and cold environments, on the other hand, still lagged considerably behind in this respect. When the cross-sectional areas are considered it is found that the sural nerve had attained a little under half the normal value after 50 days in a hot environment. The genito-femoral nerve in the same time and at a similar temperature had attained only about one-third of its normal value. In this feature the genito-femoral nerve is comparable to the warm rather than to the hot sural nerve, probably because it has been examined about three times as far from the lesion, so that the figure obtained is strongly influenced by the distally directed taper found in regenerating nerves by Quilliam (1958). This would not affect the total number of fibres present.

The relationship between the histological observations and temperature

The results so far described show that there is a relationship between the rate of maturation in the regenerating sural nerve and the environmental temperature. Within a range from about 3 to 37°C ., maturation occurred more rapidly in the warmer environments. If this is to be interpreted as a direct effect of temperature on the regenerating nerve it is necessary to show that the temperature of the nerve and of the tissues immediately surrounding it, is significantly altered by the changes in the environmental temperature. The results of measuring the subcutaneous temperatures in different environments are shown in the following table:

Environmental temperature ($^\circ\text{C}$.) ...	3-8	20-22	34-37
Subcutaneous leg temperature	31.6 ± 1.0	35.4 ± 0.4	36.6 ± 0.7
Subcutaneous foot temperature	15.3 ± 4.3	32.0 ± 0.4	34.0 ± 2.3

The mean temperatures are given with their standard deviations. They are all based on at least five observations and in all cases the differences are statistically

significant ($P < 0.01$). In these animals the intraperitoneal temperatures did not fall outside the range $37-39^{\circ}\text{C}$. in any environment, and may be taken as constant.

Subcutaneous temperatures vary considerably from time to time in relation to posture and activity (particularly in the foot), and the conditions of observation do not allow a high degree of accuracy of measurement. In the leg it is possible that some of the readings were too high, since it is difficult in an animal the size of a rat to be sure that the needle containing the thermocouple has remained subcutaneous (where the nerve is situated) and has not penetrated to some extent into underlying muscles. Errors of this kind would tend to minimize the estimated differences, since their effects would be smaller in the warmer environments where subcutaneous and deep temperatures differ least. For these reasons the measurements can do no more than show that there are significant temperature differences and give only a rough approximation to their extent. They lead to the conclusion that the temperature differences in the region of the regenerating nerve are the cause of the effects observed on the rate of maturation, because the genito-femoral nerve, regenerating in the constant intra-peritoneal temperature, failed to show them.

It should also be noted that the quite small difference in subcutaneous temperatures between the hot and the warm specimens (of the order of 1°C .) was enough to cause significant differences in the rate of maturation. It is not known how far the lower subcutaneous temperatures recorded in the foot affected the results, but it is possible that the temperature of the end-organs with which a regenerating nerve connects would affect the rate of maturation.

DISCUSSION

The experiments reported demonstrate that changes in the peripheral temperature affect the rate of maturation of regenerating nerves, a rise, within certain limits, causing acceleration and a fall, retardation. The acceleration or retardation is not confined to the early outgrowth stages of regeneration (where they were demonstrated by Deineka (1908) and Gamble (1957, 1958)), and cannot be explained as an indirect effect working through metabolic or hormonal changes which might form part of a reaction to the stress of exposure to unusual environmental temperatures. Gamble showed that the temperature effect was easily demonstrable in the caudal nerve where large subcutaneous temperature changes were produced and the present work shows that temperature differences as small as 1 or 2°C . are also effective. The temperature change produced by Deineka in the rabbit's sciatic nerve was not measured, but the situation of the nerve makes it almost certain that it was still smaller. It is probable that at nerve temperatures below 15°C . (in mammals) maturation is completely inhibited (Gamble, 1957); regeneration must also cease at some temperature above 38°C ., but precisely what happens in this upper range has not been investigated. It is probable that the optimum temperature for nerve regeneration lies at or slightly above normal body temperature as measured in the peritoneal cavity.

The fact that the influence of temperature has been exerted peripherally and not centrally in these experiments focuses attention on the peripheral rather than the central mechanisms involved. Presumably the synthesis of axoplasm in the cell-body and its passage down the axon (Weiss & Hiscoe, 1948; Weiss, 1955) was not directly affected. If alterations in the rate of these processes played any part in the

acceleration of maturation in the warmer specimens, it could have been brought about by some 'feed back' mechanism from the periphery similar to that postulated by Aitken, Sharman & Young (1947) to explain the effect of connexion with an appropriate end organ. A reduction in the viscosity of the peripheral axoplasm might also facilitate the passage of material elaborated in the cell-body.

It is questionable, however, whether it is necessary to postulate such mechanisms to explain the temperature effect. Peripheral processes such as the removal of debris, the multiplication of Schwann cells and the formation of myelin could all be accelerated by a local increase in temperature. The experiments reported give no evidence whether one of these is more affected than another. It should be noted, however, that the temperature effect was demonstrable throughout the whole period studied and that the lag of the cold specimens behind the hot and the warm seemed to increase (Text-fig. 3). This indicates that the differences were not due simply to the fact that the warmer animals had a better start and maintained their advantage. The later as well as the earlier stages of the regeneration process have been affected.

The changes which occur in the diameter of the axon alone during the maturation process were not studied in this investigation where only the total diameter of the fibre (including its sheath) was measured. The axonal changes are generally thought to be the result of the activity of the cell-body, on the basis of the work of Weiss & Hiscoe (1948) already referred to. Peripheral processes, sensitive to the local temperature, may also be important however. Hughes (1953) has shown that during the early outgrowth stages of embryonic neurites in tissue culture, pinocytosis at the ends of the growing axons may be responsible for taking up material peripherally. At later stages the suggestion of Geren & Schmitt (1954) that axonal mitochondria may be supplied by budding from the Schwann cell cytoplasm again emphasizes the possible importance of peripheral factors. Andrew (1956) remarked that the Schwann cells might act as 'nurse cells' to the extended cytoplasm of the neurone and clearly such activities could be influenced by the peripheral temperature.

In conclusion, the importance of allowing for the effect of temperature in all experiments where the rate of regeneration or of growth processes is significant, should be pointed out. The extent of the range of temperature in the extremities of mammals is often ignored. This is likely to be greater in small mammals where the ratio of surface to volume is larger, but even in man surprisingly low temperatures in the extremities have been recorded. Thus Bazett, Love, Newton, Eisenberg, Day & Forster (1948) have recorded temperatures in human arteries 'without the subjects being unduly cold or the rectal temperature particularly low' as follows: aortic, 37° C., brachial artery, 31.1° C. and radial artery 21.5° C. Clinically, nerve injuries affecting the distal segments of the limbs are of outstanding importance, and temperatures such as these might have an important effect in slowing the rate of regeneration and delaying the restitution of function.

SUMMARY

The rates of maturation of the regenerating sural and genito-femoral nerves of the rat have been studied in three different environments, hot (34–37° C.), warm (20–22° C.) and cold (3–8° C.). The stage of maturation after varying periods up to 150 days after injury by crushing was estimated from (i) the total number of

myelinated fibres present, (ii) histograms showing the fibre calibre distribution, (iii) the total cross-sectional area of the fibres in the whole nerve.

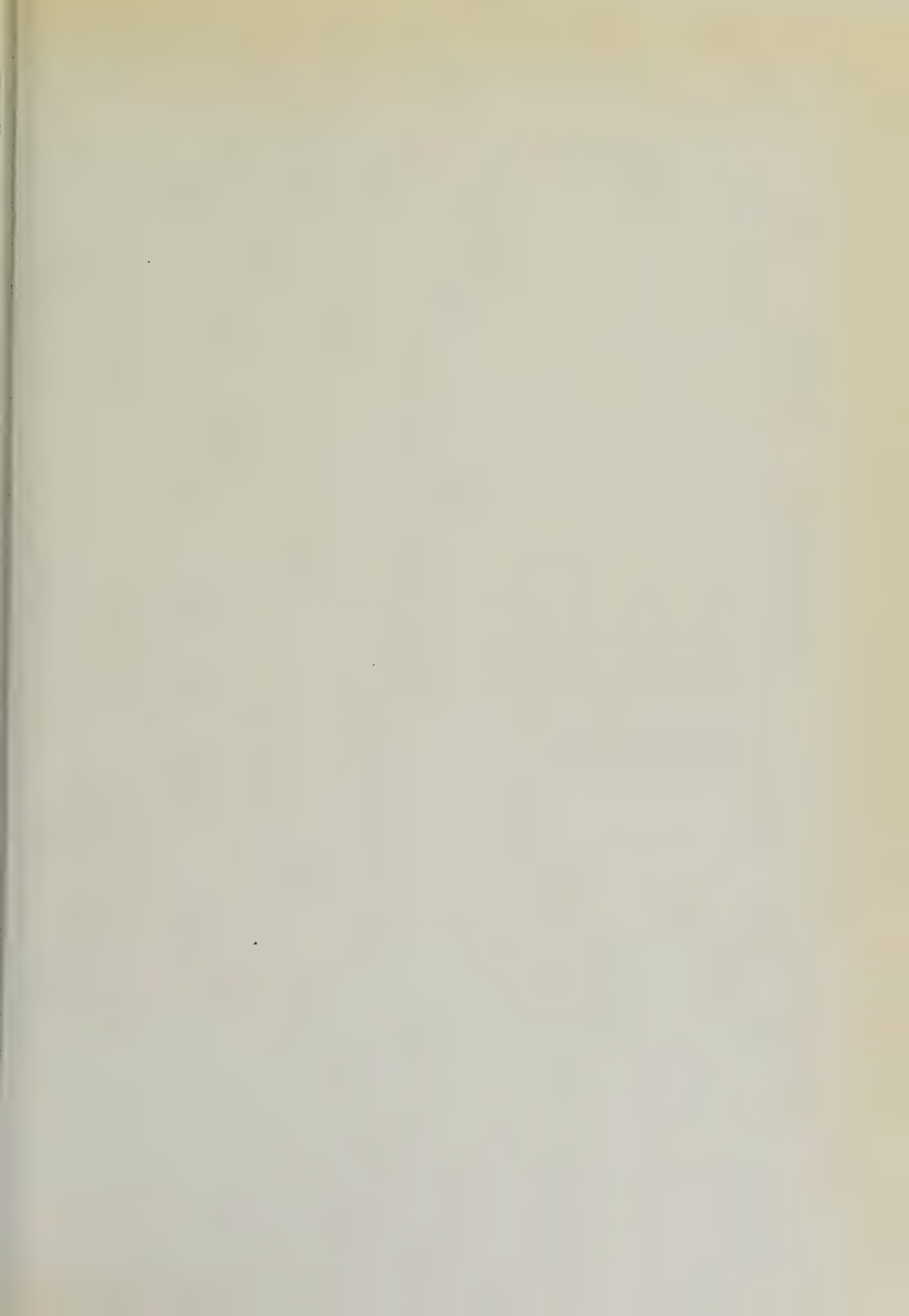
50 days after injury, significant differences in the stage of maturation reached were demonstrated in the sural nerve, between the hot and warm and between the warm and cold specimens. At all periods studied there were significant differences between the hot and the cold specimens. Smaller differences, many of which were not statistically significant, were found between the hot and warm and between the warm and cold specimens, but the uniform trend of the results suggests that a study of larger groups of specimens would show these smaller differences to be significant.

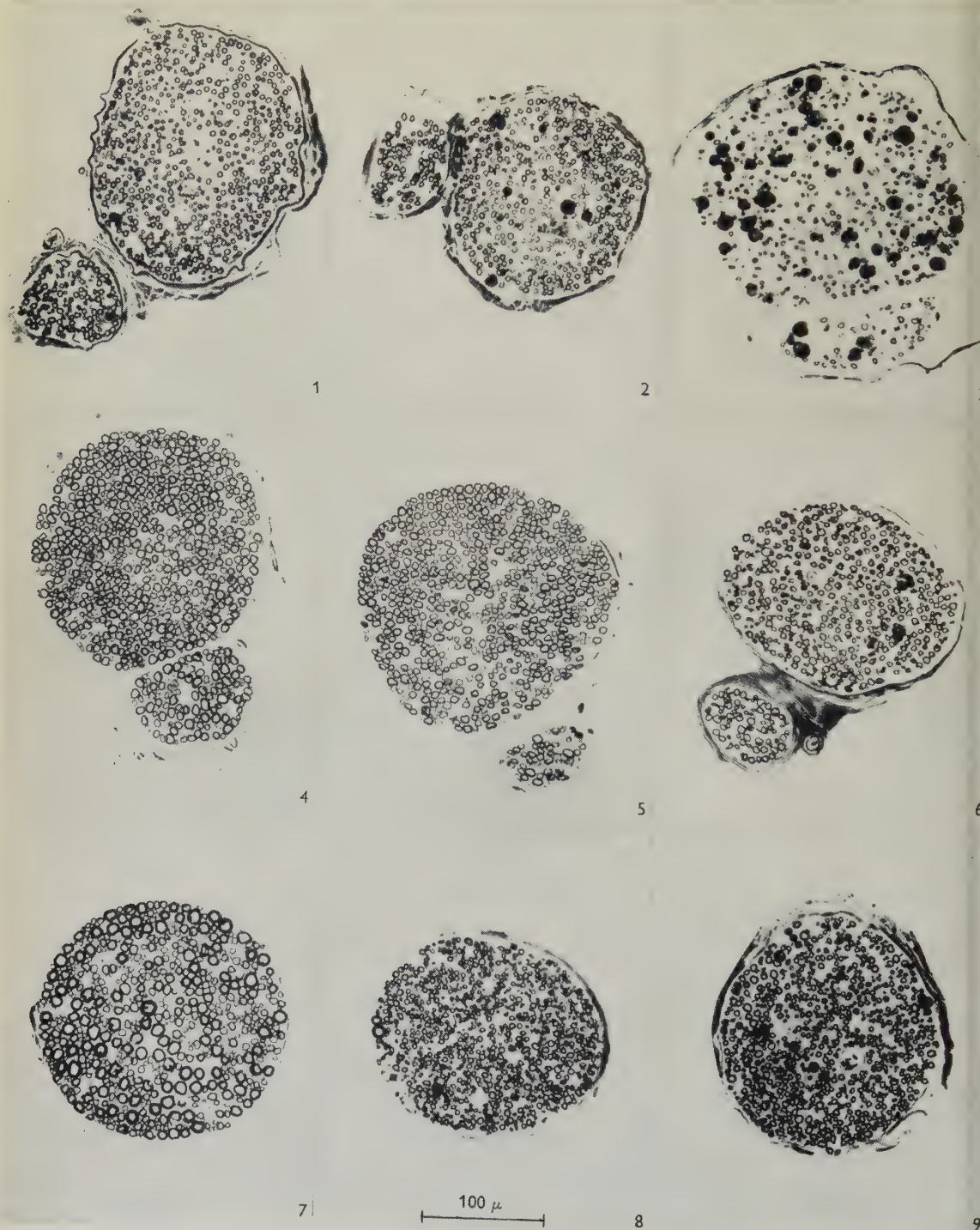
The rate of maturation in the genito-femoral nerve regenerating in the uniform temperature of the peritoneal cavity was not affected by the different environmental temperatures. It is concluded that the effect on the sural nerve must have been due to a direct effect of the temperature on the peripheral (subcutaneous) part of the nerve. The extent of subcutaneous cooling achieved in the environmental temperatures used was of the order of 1–2° C. between the hot and the warm animals and about 4° C. between the warm and the cold animals.

This paper is based on a Ph.D. Thesis (B. D. Jha, 1959) accepted by the University of London, and stored in the Library of the Senate House, London, W.C. 1. The counts and measurements made in all individual specimens are recorded in the thesis.

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EXPLANATION OF PLATE

All photographs in the Plate are of transverse sections of rat nerve stained by the Gutmann & Sanders method, all at the same magnification.

Figs. 1-3. Sural nerve after 30 days regeneration in hot (fig. 1), warm (fig. 2) and cold (fig. 3) environments.

Figs. 4-6. Sural nerve after 150 days regeneration in hot (fig. 4), warm (fig. 5) and cold (fig. 6) environments.

Fig. 7. The normal genito-femoral nerve.

Fig. 8. Regenerating genito-femoral nerve after 50 days in a cold environment.

Fig. 9. Regenerating genito-femoral nerve after 50 days in a hot environment.

THE ANATOMY OF THE SYMPATHETIC TRUNKS IN MAN

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INTRODUCTION

Even a cursory study of the anatomical descriptions of the cervical parts of the sympathetic trunks given in modern text-books or articles discloses that, now as earlier, great confusion exists with respect to terminology. This applies even to monographs and more specialized presentations. The primary cause of this confusion is the very marked variability of the trunks in the neck region, which gives wide scope for arbitrary interpretations of the arrangement; some uncertainty about the terminology and notation of other parts of the trunks also persists.

It is true that the terms to be used for the sympathetic nervous system were fixed by the International Anatomical Nomenclature Committee (*Nomina Anatomica*, Paris, 1955). This does not, however, prevent some of the individual terms being used to denote different anatomical units, and for practical reasons (such as limiting printing costs) comprehensive explanations could not always be given in the annotations to the Parisian *Nomina Anatomica*. As one of the three members of the Sub-Committee responsible for the nomenclature of the peripheral nervous system, I wish to define more exactly my views on the terminology adopted for the sympathetic trunks. I also take this opportunity of revising a few terms I used in certain papers published some twenty years ago.

In *Nomina Anatomica* the term *truncus sympathicus* is followed by the names of its ganglia, *ganglia trunci sympathici*, as well as of its connecting *rami interganglionares*. But, also under the heading *ganglia trunci sympathici*, the term *ganglia intermedia* is used to denote ganglia on the *rami communicantes* and certain ganglia on the trunks in the *rami interganglionares* between the other ganglia—namely the *ganglion cervicale superius*, *ganglion cervicale medium*, *ganglion cervicothoracicum* (*s. stellatum*), *ganglia thoracica*, *ganglia lumbalia*, *ganglia sacralia*, and *ganglion impar*. The term *ganglion vertebrale* is listed under the heading *ganglion cervicale medium*, without further commentary.

The main object of the present paper is to define those anatomical units to which the above terms should be applied.

CERVICAL REGION

The cervical parts of the sympathetic trunks differ essentially from the other parts, because their segmentation has become so obliterated, owing to fusion and division of the segmental ganglia, that it has been necessary to give each ganglion a special name. In many text-books it is stated that the individual cervical ganglia correspond to certain fixed segments, as evidenced by their macroscopically demonstrable connexions with certain spinal nerves through the communicating rami. In the

cervical region it is not possible to analyse the connexions between the spinal nerves and the trunk solely by macroscopic dissection (Wrete, 1934*a*) because the connexions consist partly of spinal-nerve branches to the prevertebral muscles, which are joined to the grey communicating rami in a highly complicated way (Fig. 1).

However, by microscopic studies of embryos and foetuses (Wrete, 1934*b*) it was possible to make a segmental analysis of the cervical sympathetic trunks (Fig. 2).

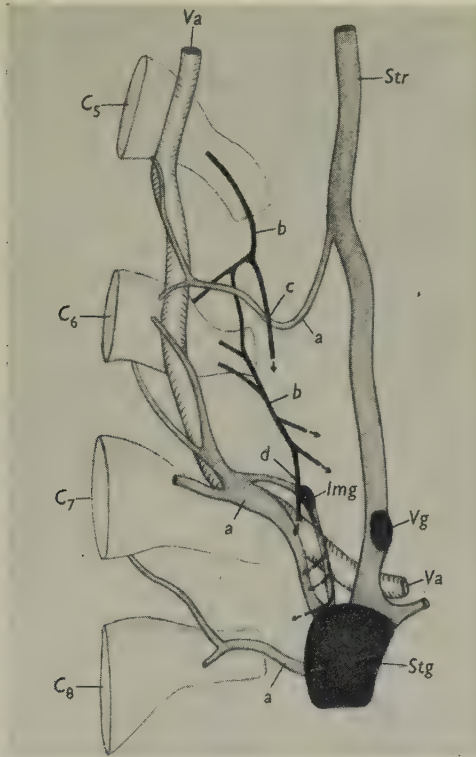


Fig. 1. Human foetus, 39.6 mm. Profile reconstruction from the left half of the body, seen from the median plane. *C*₅, *C*₆, etc., parts of cervical spinal nerves; *Va*, vertebral artery; *Vg*, vertebral ganglion; *Str*, sympathetic trunk; *Stg*, stellate ganglion; *a*, grey communicating ramus; *b*, spinal-nerve branch to prevertebral muscles (the arrows mark the terminal branches of the spinal-nerve branches running towards the muscles); *c* and *d*, junction of *a* and *b* (the broken line denotes part of a muscle branch which follows a grey communicating ramus); *Img*, intermediate ganglion on this communicating ramus.

This was done by investigating the embryonic segmental parietal arteries, and particularly the grey communicating rami accompanying them, but unfortunately little attention has been paid to these observations. These communicating rami, which are present only in the lower part of the cervical region and the uppermost part of the thoracic region, are characterized by their division into two branches, one to the segmental artery corresponding to the spinal nerve, and one to the cranial artery in immediate succession; I therefore introduced the term '*rami communicantes grisei bipartiti*' for these rami. From a linguistic point of view, the term is

not particularly appropriate, and it does not seem to have become generally accepted. (I have, in fact, seen it only in two text-books.) Consequently, in view of the fork-like mode of branching of the nerves in question, I now suggest that they be named '*rami communicantes grisei bifurcati*'.

Throughout the literature the term superior cervical ganglion is used to denote the most cranial of the ganglia on the cervical part of the sympathetic trunk,

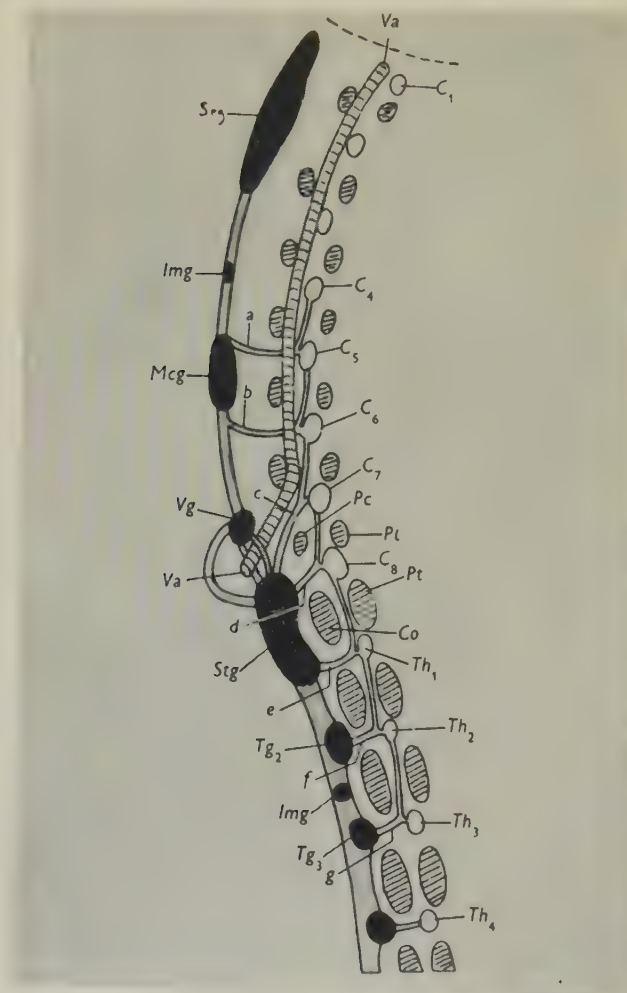


Fig. 2. Schema showing the course of the *rami communicantes grisei bifurcati* in man. Left sympathetic trunk seen from the lateral view. The transverse process and ribs are sawn through, and the lateral parts of the cut surface are removed (broken lines indicate the cut surfaces). *Pc*, costal process of 7th cervical vertebra; *Pl*, lateral process of same vertebra; *Co*, neck of first rib; *Pt*, transverse process of first thoracic vertebra; *Va*, vertebral artery; *C*₁, *C*₄, etc., cervical spinal nerves; *Th*₁, etc., thoracic spinal nerves; *Mcg*, middle cervical ganglion; *Scg*, superior cervical ganglion; *Stg*, stellate ganglion; *Vg*, vertebral ganglion; *Tg*₂, etc., thoracic ganglia; *Img*, intermediate ganglion; *a*, *b*, deep *rami communicantes*; *c*, vertebral nerve; *d*, *e*, *f*, *g*, caudal *rami communicantes grisei bifurcati*.

irrespective of variations in shape, size and position. There is no unanimity about these variations.

In most text-books the term stellate ganglion is used to denote that ganglion formed by fusion of the lowest ganglion on the cervical trunk and one or more (usually one to four) of the adjacent thoracic ganglia. These most cranial thoracic ganglia can be identified with the help of the *rami communicantes grisei bifurcati*. The upper part of the stellate ganglion was formerly known as the inferior cervical ganglion. This term has sometimes given rise to confusion, since it has also been used, incorrectly, for a ganglion cranial to the subclavian artery. The fact that the term has been discarded in *Nomina Anatomica* is justified by the fact that the inferior cervical ganglion appears only exceptionally as an independent formation. The part of the stellate ganglion which corresponds to it is characterized by its position directly caudal to the subclavian (and the root of the vertebral) artery, and by the fact that it gives off two *rami communicantes grisei bifurcati* (Wrete, 1934*b*); the cranial ramus, which is developed along the 7th cervical segmental artery and is named the vertebral nerve (*nervus vertebralis*), joins the spinal nerves C_7 and C_6 ; the caudal ramus, developed along the 8th cervical artery, joins C_8 and C_7 . It is not unusual to find a constriction on the stellate ganglion, marking the borderline between the parts representing the inferior cervical ganglion and the 1st thoracic ganglion.

The suggestion put forward by Lazorthes & Cassan (1939), and adopted by Guerrier (1944), that the term cervicothoracic or stellate ganglion should include the vertebral ganglion cranial to the origin of the vertebral artery is not warranted, as Mitchell (1953) has justifiably stressed. In my opinion this also applies to a similar suggestion put forward by Axford (1927-8) and Woollard & Norrish (1933), i.e. that it should also include this ganglion, although they failed to differentiate the vertebral from the middle cervical ganglion.

The greatest divergences in the nomenclature are encountered with respect to the part of the sympathetic trunk between the superior cervical ganglion and the stellate ganglion. In most descriptions only one ganglion is mentioned, namely the middle cervical ganglion. Some authors state it is situated relatively high up and others place it relatively low down, close to the root of the vertebral artery. Axford (1927-8) refers to high and low middle cervical ganglia. When two ganglia were present, they were described by van den Broek (1908) as a middle cervical ganglion split into two parts. In an earlier publication (Wrete, 1934*b*) I also used this less appropriate nomenclature.

Mannu (1914) denoted all ganglia between the superior cervical ganglion and the inferior cervical ganglion as intermediate ganglia; he distinguished as particularly characteristic a superior one, the thyroid ganglion, and an inferior one, close to the subclavian artery, the vertebral or subclavian ganglion. In some modern text-books of anatomy an attempt has been made to clarify the terminology by denoting the superior, generally larger ganglion as the middle cervical ganglion, and the inferior one as the intermediate cervical ganglion (e.g. Jonnesco, 1923; Hovelacque, 1927; Kuntz, 1946; Brodal, 1948; White & Smithwick, 1952). Matsui (1925-6) has used the term intermediate ganglion as a synonym of middle cervical ganglion.

On the basis of a study of the cervical sympathetic trunks in 120 fetuses and

newborn infants, Laubmann (1931) set up a schema with five basic types (Fig. 3), some of which are stated to be more and others less common. Since there is reason to presume a postnatal reshaping of the cervical sympathetic trunks (Wrete, 1934*b*), the incidence figures given by Laubmann may not be fully applicable to adults, nor does this schema cover all the variants which may occur. Despite these drawbacks, Laubmann's schema is of great value. In all the main types and subtypes the lower of the two interjacent ganglia, lying slightly cranial to the origin of the vertebral artery from the subclavian artery, is indicated. I also found this ganglion to occur with great regularity in extensive macroscopic and microscopic studies of the cervical sympathetic trunks and their segmental branches to the spinal nerves in my series of 41 embryos, fetuses, newborn infants and adults. Laubmann suggested the term vertebral ganglion for this structure, and middle cervical ganglion for the larger ganglion lying more cranially. The use of these two terms, with which I concur, has the great advantage over the use of only one term in that it recalls the fact that segmentally differing parts of the trunk are involved. As Mitchell (1953) has correctly emphasized in his excellent monograph on the autonomic nervous system, the term vertebral ganglion is preferable to intermediate ganglion, used by a number of modern authors (*vide supra*), since the ganglion 'is related to and helps to supply the vertebral artery, and because the term intermediate ganglion is now invariably applied to ganglia on the rami communicantes or ventral nerve roots'. Mitchell's view is based on dissections of about 100 specimens.

Substitution of the term vertebral ganglion for intermediate ganglion allows the latter to be reserved for the small ganglion, or ganglia, sometimes present between the ganglia listed, both in the cervical and other parts of the sympathetic trunk (Wrete, 1935, 1941*a, b*, 1943, 1951).

To understand the anatomical variations of the cervical sympathetic trunks it is necessary to study their ontogenesis. I shall, therefore, give a brief account of my earlier studies (Wrete, 1934*b*), since they illustrate particularly well certain problems of nomenclature. In describing the results I shall use the terminology suggested above, and thus distinguish between middle cervical ganglion and vertebral ganglion.

In a 10 mm. human embryo, where the cervical sympathetic trunk appears as a homogeneous ganglionated cord, immediately cranial to the root of the vertebral artery, the primordium of the vertebral ganglion appears as a part separated from the other, more cranial part by a constriction. At this stage the *rami communicantes grisei bifurcati* have started to develop. In a 15.4 mm. embryo the cranial part of the ganglionated cord, which has a well-defined constriction in the middle, has been displaced some distance cranially, and is now separated from the vertebral ganglion by a distinct interganglionic ramus. In a 29 mm. foetus, this ramus has become further elongated. In both the latter stages, the caudal part of the cranial ganglionated mass gives rise to the most cranial of the *rami communicantes grisei bifurcati*, corresponding to the 6th cervical artery, showing that this caudal end consists of the cranial part of the 6th cervical segment of the trunk, whereas the vertebral ganglion comprises the caudal part of the segment.

In specimens from later stages of foetal life, as well as in preparations from newborn infants and adults, marked individual variations are seen. In some cases development of the ganglia has been arrested at the aforementioned stage. In

other cases development has proceeded yet another step, so that the cranial ganglionated mass has been divided into an upper, superior cervical ganglion, and a middle cervical ganglion caudal to it. This produces a state corresponding to the most common type in Laubmann's schema (type I), which comprised about one-third of all the cases in his material. I have named this the *basic type* (Fig. 4A). Here, the chief characteristic of the middle cervical ganglion is that it gives off one or two *rami communicantes grisei bifurcati*. The superior cervical ganglion then consists of the four most cranial segments of the trunk, the middle cervical ganglion comprises the 5th and upper part of the 6th, and the vertebral ganglion is formed from the lower part of the 6th.

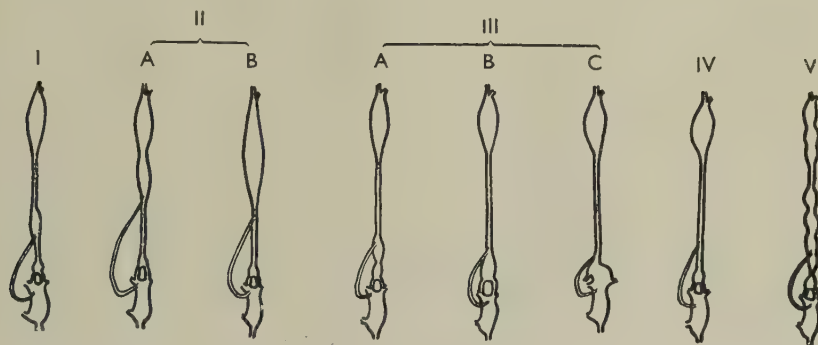


Fig. 3. Laubmann's type schema.

In other cases the development of the cervical part of the trunk is arrested at a still earlier stage, and the course of the *rami communicantes grisei bifurcati* shows that the cranial ganglion, generally termed the superior cervical ganglion, is actually the result of fusion of this ganglion and the middle cervical ganglion. Consequently this anomalous ganglion should rightly be denoted as a *medio-superior cervical ganglion* (*ganglion cervicale medio-superius*) (see Fig. 4B). This type of sympathetic trunk, corresponding largely to Laubmann's type II, is—as he pointed out—a primitive type. The ganglion is sometimes short and thick, and in such cases the *rami communicantes grisei bifurcati* are given off from the interganglionic ramus at a variable distance caudal to the ganglion (Fig. 4C): its closest analogue in Laubmann's schema is type IV which comprised about one-fourth of all his cases.

In still other cases the course of the *rami communicantes grisei bifurcati* shows conclusively that the 5th and 6th segments of the trunk have remained in a low position, forming a ganglion which should be denoted as a *medio-vertebral cervical ganglion* (*ganglion cervicale medio-vertebrale*) (see Fig. 4D). In this type a superior cervical ganglion is present which corresponds to the ganglion of the basic type with the same name. This anomaly is represented by type III in Laubmann's schema; he found it to be more common than type II, but less common than type IV.

It is obviously a misleading simplification to use invariably the term superior cervical ganglion (*ganglion cervicale superius*) for the most cranial ganglion of the cervical part of the trunk. Moreover, in the basic type and the medio-vertebral type, this most cranial ganglion is not implicated in the sympathetic supply to the vertebral plexus and the arm, whereas the medio-superior ganglion type is involved

in this supply. This circumstance should be borne in mind in planning surgical denervation of the blood vessels of the arm.

The communicating rami visible in Fig. 4 have long been known as the deep communicating rami (*rami communicantes profundus*). They are the most cranial of the groups of nerves that I have called *rami communicantes grisei bifurcati*, situated cranially to the subclavian artery and the root of the vertebral artery. I found them to be absent in only 4 of 55 sides (Wrete, 1934*b*). Very often only the cranial one was lacking, whereas absence of the caudal one alone was uncommon. These rami

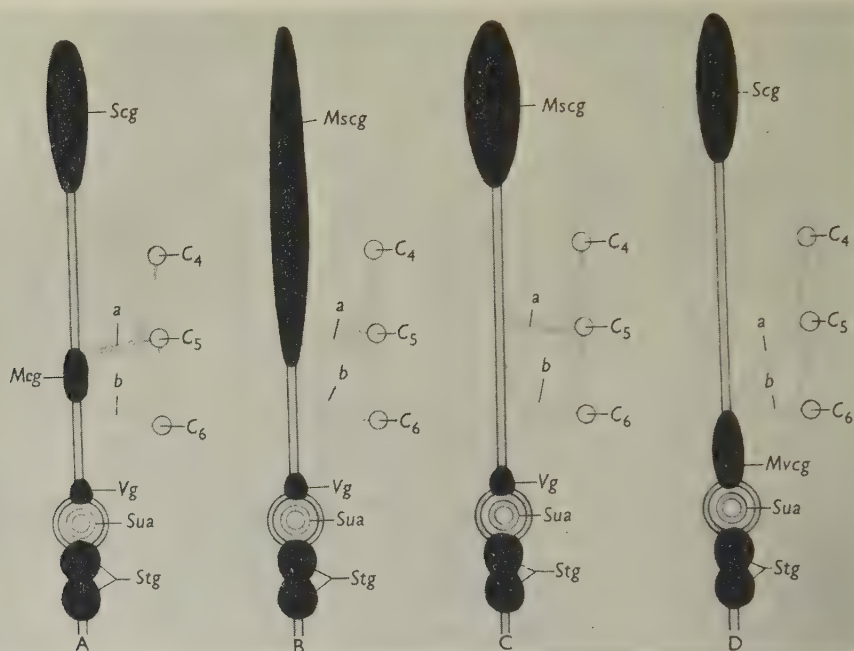


Fig. 4. Type schema set up on the basis of the segmental structure of the cervical sympathetic trunk. C_4 , C_5 , C_6 , cervical spinal nerves; *a*, *b*, rami communicantes grisei bifurcati; *Sua*, subclavian artery; *Scg*, superior cervical ganglion; *Mcg*, middle cervical ganglion; *Mscg*, medio-superior cervical ganglion; *Mvcg*, medio-vertebral cervical ganglion; *Stg*, stellate ganglion; *Vg*, vertebral ganglion.

are easily identified by the fact that they pierce the longus colli muscle on their course from the sympathetic trunk to the spinal nerves, which they join close to the vertebral artery. They may be united with branches of the spinal nerves passing to the prevertebral muscles (Wrete, 1934*a*) but, despite this, they should be identifiable even at operation.

Laubmann's type V, which is uncommon and must be regarded as a fairly gross developmental disturbance, probably appears only in association with anomalies in development of the segmental arteries (Wrete, 1934*b*). I found that such vascular anomalies could be associated with marked variations in the anatomy of the cervical sympathetic trunk. It is therefore essential, in a study of the normal anatomy of this part of the trunk and of the stellate ganglion, to disregard cases with vascular anomalies.

THORACIC, LUMBAR AND SACRAL REGIONS

At first sight the terminology of the parts of the sympathetic trunk caudal to the cervical region seems to present no difficulties, because the thoracic region may have 12 ganglia, the lumbar region 5, and the sacral region 5, i.e. the same number as the segments. When this applies, the ganglia can simply be denoted as the 1st–12th thoracic ganglia, 1st–5th lumbar ganglia and 1st–5th sacral ganglia. In actual fact, however, their number is highly variable. Thus there may be 9 or 13 ganglia in the thoracic region, 1 or 7 in the lumbar region, and 2 or 6 in the sacral region, and then difficulties arise in naming the individual ganglia. If each ganglion were joined by one or several communicating rami to a single spinal nerve, it could be given the name of the relevant nerve, but this is the case only in the upper part of the thoracic region. Attempts have been made to number the ganglia according to the spinal nerve to which each is joined by the communicating rami (van den Broek, 1908), by its white communication ramus (Zuckerman, 1937–8), or by its grey communicating ramus (Langley, 1896; Ranson & Billingsley, 1918; Sheehan & Pick, 1942–3). The last-mentioned authors have dealt fairly extensively with the question of terminology in connexion with a study of the communicating rami in Rhesus monkeys. It is more convenient to use the grey instead of the white rami, since, as Sheehan & Pick have pointed out, ‘the fibres entering the sympathetic trunk through one white ramus may be distributed to 9–10 successive ganglia and, furthermore, certain ganglia receive two and even three white rami from several spinal nerves’. However, the use of the grey communicating rami is also associated with drawbacks. It is not always possible to distinguish the white and grey rami from each other macroscopically, since they are often mixed, and Sheehan & Pick admitted that histological examination was often necessary. They had to adopt arbitrary rules in order to bring about uniformity in the descriptions of the various dissections and to permit accurate comparison, even if microscopic studies were available. The same principles were applied by Pick & Sheehan (1946) in a later study of the communicating rami in man.

The procedure of Sheehan & Pick is applicable only in the extremely rare situation when all communicating rami are examined histologically to determine whether they are of the white or grey type. Even then difficulties occur. On macroscopic dissection the procedure can scarcely be used, in view of the great difficulty of distinguishing between the two kinds of rami. Soulié (1901) has gone so far as to state that, in man, this is possible only in exceptional cases; van den Broek (1908) came to the same conclusion in a study of a large number of mammalian species. In fresh material the difference could occasionally be observed, but in preserved material no difference was detectable.

In my opinion, it is more appropriate to choose another procedure, which facilitates exact terminology, and which is applicable even when deviations are present from the simple basic pattern of 12 thoracic, 5 lumbar and 5 sacral ganglia. In other words, the terminology should be entirely independent of the communicating rami, in view of their highly variable course, and the difficulties in distinguishing between them.

On a wax reconstruction, according to Born’s method, of the sympathetic trunks

in a 10.3 mm. embryo (Wrete, 1930), the parts of the trunk caudal to the cervical region are distinctly segmented, and the ganglia lie in the spaces between the segmental, parietal branches of the aorta. These subsequently give rise to the 1st to 11th posterior intercostal arteries, the subcostal artery and the lumbar arteries, as well as lateral branches of the median sacral artery. Not only in human embryos and foetuses, but also in the newborn and adult, all these arteries cross the sympathetic

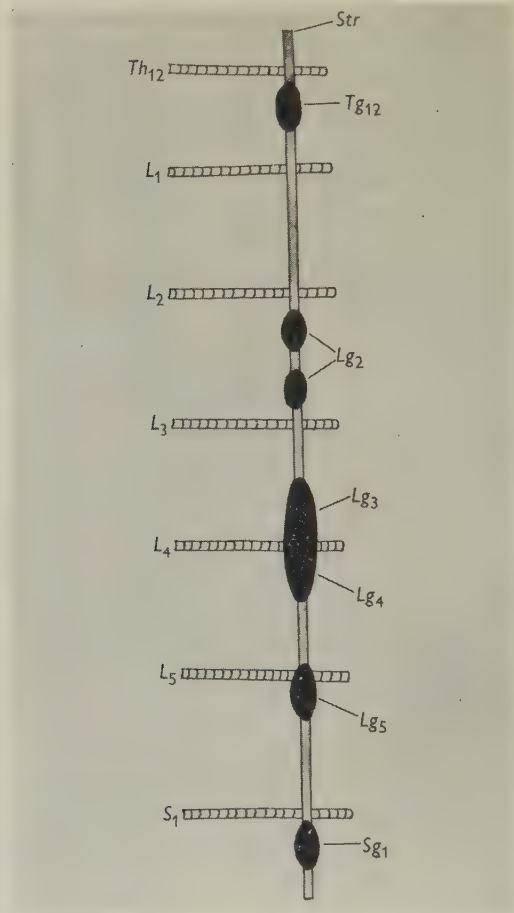


Fig. 5. Schema showing the thoracic, lumbar and sacral regions of the sympathetic trunk. Th_{12} - S_1 , segmental parietal arteries; Str , sympathetic trunk; Th_{12} , L_1 - L_5 , S_1 , segmental parietal arteries; Tg_{12} , Lg_2 - Lg_5 , Sg_1 , thoracic lumbar and sacral ganglia of the sympathetic trunk.

trunk and lie close to it. This permits convenient, exact division of the trunk within its segments. Thus the term 4th thoracic ganglion can be used to denote the ganglion lying in that part of the trunk between the 4th and 5th intercostal arteries, the 12th thoracic ganglion is the ganglion lying between the subcostal and the 1st lumbar arteries, and so on.

Finally, it is reiterated that *rami communicantes grisei bifurcati* are present in the

three or four uppermost thoracic segments (Wrete, 1934a) as they have developed along the segmental arteries. They can therefore be used for an analysis of the stellate ganglion, to determine the number of thoracic ganglia in it.

Application of the terminology I have suggested is illustrated schematically in Fig. 5 which shows some segmental arteries ($Th_{12}-S_1$), as well as a corresponding length of the sympathetic trunk. The uppermost of the ganglia is the 12th thoracic ganglion. The 1st lumbar ganglion is lacking, and the 2nd is split into two parts. The 3rd and 4th are fused into a common ganglion. The caudal one next in order should be termed the 5th lumbar ganglion, since only a minor part of it projects above the 5th segmental artery; it is not necessary in such a case to describe the upper part of the ganglion as a caudally situated part of the 4th lumbar ganglion. Obviously a segmental artery may occasionally have an anomalous course and, in this event, it should be possible to determine approximately how it would have run normally and to number the relevant ganglion accordingly.

SUMMARY

The individual ganglia on the sympathetic trunks in man have the following characteristics.

The cervical part of the trunk generally has four typical ganglia, the superior cervical ganglion, the middle cervical ganglion, the vertebral ganglion, and the stellate ganglion of which the lower part belongs to the thoracic part of the trunk.

The middle cervical ganglion (*ganglion cervicale medium*) gives off a *ramus communicans griseus bifurcatus* to the 6th and 5th cervical nerves, and sometimes one to the 5th and 4th as well. The ganglion cranial to it is the superior cervical ganglion (*ganglion cervicale superius*). These two ganglia are often fused into what should be denoted as a medio-superior cervical ganglion (*ganglion cervicale medio-superius*).

The vertebral ganglion (*ganglion vertebrale*) is relatively small, and lies directly cranial to the subclavian and the root of the vertebral artery. It is not infrequently fused with the middle cervical ganglion, thus forming a medio-vertebral cervical ganglion (*ganglion cervicale medio-vertebrale*).

The medio-superior and medio-vertebral ganglia are common variants.

The upper part of the stellate ganglion (*ganglion stellatum s. cervicothoracicum*) only exceptionally appears as an independent formation; it was formerly known as the inferior cervical ganglion (*ganglion cervicale inferius*). Characteristically it is joined respectively to the 8th and 7th cervical nerves and to the 7th and 6th cervical nerves through the caudal and cranial rami of a *ramus communicans griseus bifurcatus*. The lower part of the stellate ganglion usually consists of the 1st thoracic ganglion, which can be identified by its connexion with Th_1 and C_8 through a *ramus communicans griseus bifurcatus*. The 2nd and 3rd thoracic ganglia, and even the 4th, may form part of the stellate ganglion. These ganglia can be identified by the communicating rami of a similar kind given off by them, whereas such rami are lacking in the segments caudal to them.

The above statements about the cervical sympathetic trunk ganglia are valid only when the cervical segmental arteries are normally developed.

Below the stellate ganglion, each ganglion should be named after the space between the segmental, parietal arteries (e.g. intercostal arteries, lumbar arteries)

in which it is situated. For example, the ganglion lying between the 3rd and 4th lumbar arteries is denoted as the 3rd lumbar ganglion.

The term intermediate ganglion (*ganglion intermedium*) should be reserved for the small, inconstant ganglia situated in the internodal rami (*rami internodiales*) between the ganglia listed above, at any site in the trunk, as well as in the communicating rami or in the main trunks or roots of the spinal nerves.

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NUCLEAR POPULATION CHANGES IN DEGENERATING NON-MYELINATED NERVES

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INTRODUCTION

Section of a myelinated nerve trunk is followed by an increase in the number of mitoses in the peripheral stump (Delcourt, 1939) and an increase in its nuclear population (Abercrombie & Johnson, 1946; Thomas, 1948), partly if not entirely as a result of these nuclear divisions. It might reasonably be expected that changes of a similar nature would be found during degeneration in so-called non-myelinated nerves. Changes in nuclear population may be followed by a study of nuclear counts or of the frequency of mitoses, the latter being a measure of the increase in endogenous population. Joseph (1947, 1950) found no significant increase in the number of nuclei in the degenerating anterior mesenteric nerve of the rabbit. On the other hand, Rexed & Fredriksson (1956) found a marked increase in the frequency of mitoses in the 'very poorly myelinated' thoracic vagus nerve of the guinea-pig which reached a maximum on the fourth day of degeneration. These authors also examined the splenic nerve of the rabbit and believed that the frequency of mitoses was similar, without, however, quantitative verification. More recently, Abercrombie, Evans & Murray (1959) found a significant increase in the nuclear population of the abdominal vagus of the rabbit after 5 and 10 days' degeneration. The anterior mesenteric, splenic and abdominal vagus nerves of the rabbit are almost totally non-myelinated (Simpson & Young, 1945; Rexed & Fredriksson, 1956; Evans & Murray, 1954).

The apparent discrepancy between these results made it desirable to investigate the frequency of mitoses in the degenerating anterior mesenteric nerve of the rabbit and to re-examine the previous findings of one of the authors (J. J.).

MATERIAL AND METHOD

In three adult rabbits the anterior mesenteric ganglion was removed to produce degeneration in the anterior mesenteric nerve. Four days later, at 10 a.m., 1 ml./400 g. body weight of a 0.12% aqueous solution of colchicine was injected intraperitoneally. Six hours later the animal was killed and the anterior mesenteric nerve removed. Three other adult rabbits with intact anterior mesenteric ganglia and nerves were killed 6 hr. after the administration of colchicine.

The specimens were fixed in Bouin's fluid, embedded in paraffin and sectioned at 7 μ . Initially sections were cut transversely but the majority were cut longitudinally and counts were made on these. Some sections were stained with haematoxylin and eosin and others with Weigert's haematoxylin and picric acid. The latter was found more convenient for counts of mitoses. Sections were examined at a magnification of 1100 diameters. Fields were taken at random and then a systematic coverage of

the surrounding fields made until between 2500 and 3000 nuclei in longitudinal section had been counted for each nerve. No distinction was made between the various cell types and areas were not measured.

RESULTS

The number of mitoses observed in each nerve and group of nerves is given in Table 1. The five mitoses observed in non-degenerating nerve were all in metaphase. Of the forty-nine mitoses observed in degenerating nerve forty-six were in metaphase and one each in prophase, anaphase and telophase.

Table 1. *Frequency of mitoses in non-degenerating and degenerating anterior mesenteric nerves*

Animal no.	Days of degeneration	No. of nuclei	No. of mitoses	% mitoses
N2	0	2564	2	0.08
N3	0	2941	2	0.07
N6	0	2695	1	0.04
N2, 3 and 6	0	8200	5	0.06
N1	4	2936	14	0.48
N4	4	2560	19	0.74
N5	4	2766	16	0.58
N1, 4 and 5	4	8262	49	0.59

The difference in mitotic frequency between the non-degenerating and degenerating nerves is highly significant ($\chi^2 = 39.2$, D.F. 1, $P < 0.005$).

DISCUSSION

The increased frequency of mitoses during degeneration in the anterior mesenteric nerve of the rabbit supports the conclusions of Rexed & Fredriksson (1956) and Abercrombie *et al.* (1959), and the general thesis that section of peripheral nerve is followed by an increase in the frequency of mitoses and in the nuclear population. In the light of these observations it was thought that a re-examination should be made of the findings of Joseph (1947, 1950) that in the anterior mesenteric nerve no significant increase in nuclear population occurred during degeneration.

The anterior mesenteric nerve is a typical peri-arterial autonomic nerve consisting of a number of bundles forming a plexus round the anterior mesenteric artery. The bundles vary in size and obliquity so that a section transverse to the long axis of the artery does not give transverse sections of all the bundles and meaningful complete counts cannot be made. For these reasons a sampling method was used and counts were made on three satisfactory bundles from each nerve. The areas of these bundles were measured in order to obtain the number of nuclei per 10,000 μ^2 . Table 2 shows the mean areas of the bundles and their sources. An analysis of variance in which the variance due to regression of area on nuclear count is removed demonstrates a highly significant difference in bundle area between the data for normal and degenerating nerve from Joseph (1947) ($F = 9.37$, $f_1 = 1$, $f_2 = 26$, $P = 0.005$). If all data for degenerating anterior mesenteric nerve (Joseph, 1947, 1950) are

pooled ($F = 1.22, f_1 = 52, f_2 = 3, P > 0.25$) then the difference from normal nerve is significant ($F = 5.28, f_1 = 1, f_2 = 71, 0.025 > P > 0.01$).

Given that the nerve bundles swell in the degenerating anterior mesenteric nerve as in other peripheral nerves (Abercrombie & Johnson, 1946) it follows that the estimation of nuclei per 10,000 μ^2 as an index of nuclear population changes is inappropriate. A better measure would have been the number of nuclei in a complete section (Joseph, 1948) allowing volume changes to be ignored, but as mentioned above the anatomy of the anterior mesenteric nerve precluded this. The best index available for this nerve is probably the actual number of nuclei counted.

Table 2. *Mean areas of nerve bundles* (Joseph, 1947, 1950)

Source	Days of degeneration	Mean area in 10,000 μ^2	Percentage increase on O-day
1947, Table I	0	1.003	0
1950, Table II	7	1.321	32
1950, Table II	14	1.213	21
1947, Table II	21	1.342	34
1950, Table I			

A similar analysis of variance, removing variance due to regression of count on area, demonstrates a significant difference in bundle count between the data for normal and degenerating nerve from Joseph (1947) ($F = 5.9, f_1 = 1, f_2 = 26, 0.025 > P > 0.01$). Comparing the normal with the pooled data for degenerating anterior mesenteric nerve (Joseph, 1947, 1950), $F = 1.92, f_1 = 1, f_2 = 71$, which is not significant at the 5% level, being so only at the 17% level, i.e. the chance of a variance ratio of this magnitude resulting from random sampling from a common population is 1 in 6. It will be remembered that to say a difference is not significant means only *not proven* and that if $P = 0.17$ for the null hypothesis of no difference, then $P' = 1 - P = 0.83$ for the alternative hypothesis of some difference, i.e. in five cases out of six random sampling from a common population would result in a smaller variance ratio than that obtained. These results are certainly more in favour of nuclear multiplication than its absence, and when coupled with the admonitions of Snedecor (1956) and the evidence cited above lead to the conclusion that such multiplication does occur in the degenerating anterior mesenteric nerve.

Table 3. *Nuclear counts during degeneration* (Joseph, 1947, 1950)

Source	Days of degeneration	Nuclear count	Percentage increase on O-day
1947, Table I	0	1034	0
1950, Table II	7	1082	5
1950, Table III	14	1130	9
1947, Table II	21	1232	28
1950, Table I	21	1418	

It remains to assess the amount of increase. Our best estimator of nuclear population is the total number of nuclei counted for each period of degeneration. In Table 3 these counts together with estimates of percentage increase are presented. Too much importance should not be attached to the actual numbers in these estimates in view of the variation due to the nature of the nerve and the sampling method necessary.

However, Joseph (1947) stated that the nuclear population in a 21-day degenerating non-myelinated nerve is not greater, *by a factor of two*, than in the normal nerve. This observation is confirmed by the reconsideration of the data and supported by the findings of Abercrombie *et al.* (1959) in 25-day degenerating nerve. The available evidence indicates that the nuclear population would have passed its peak by this time and it is believed that the above estimates of 7-day and 14-day populations are too low.

It is concluded then that section of a nerve is followed by swelling of the peripheral stump; an increase in the frequency of mitoses and an increase in the nuclear population. The one exception to this statement appears to be the corneal nerves where the process may be inhibited by mucopolysaccharides (Rexed & Rexed, 1951).

The factors determining the amount of increase in nuclear population remain a matter for speculation. It has been suggested that the correlation of population increase with fibre diameter (Thomas, 1948; Joseph, 1950) is less important than with the total mass of nerve fibre destroyed and the initial density of population (Abercrombie & Santler, 1957). None of these hypotheses fit all the facts. In particular the hypotheses of Abercrombie & Santler (1957) were not supported by their observations on the sural nerve. Exceptional swelling during degeneration and high non-myelinated fibre content were cited as possible reasons for the aberrant results from that nerve. If the stimulus to cell multiplication is chemical, coming by diffusion from the autolysing nerve fibres (Abercrombie & Johnson, 1946), it most probably originates in either the myelin sheath or the axon. It has been demonstrated (Wendell-Smith & Williams, 1958) that fibres in the sural nerve have thinner myelin sheaths than the fibres in the nerve to the medial head of gastrocnemius, so that measurements failing to differentiate between the relative sizes of the axon and the myelin sheath in different nerves give an incomplete picture. The picture is also incomplete in respect of non-myelinated fibres. Their quantitative histology has still to be determined.

SUMMARY

1. The frequency of mitoses in the so-called non-myelinated anterior mesenteric nerve of rabbits, which were given colchicine prior to biopsy, has been studied in intact nerves and the peripheral stumps of sectioned nerves. The increase in degenerating nerves is highly significant.

2. A re-examination of the data of Joseph (1947, 1950) shows that there is a significant difference in bundle area between the non-degenerating and degenerating anterior mesenteric nerves and that the difference in the actual counts of nuclei, while less significant, is in favour of an increase in the number of nuclei in degenerating nerves.

3. These results support the generalization that the peripheral stump of a sectioned nerve swells and exhibits an increase in the frequency of mitoses and in the nuclear population. The extent of the increase varies with the type of nerve and is least in a non-myelinated nerve.

We wish to thank Miss J. Townsend for her technical assistance.

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AN EXPERIMENTAL STUDY OF THE ORGANIZATION OF THE RETICULOENDOTHELIAL SYSTEM IN THE RED PULP OF THE SPLEEN*

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The red pulp of the spleen is a concentration of reticular cells organized as sinal blood vessels and as cellular cords separating these vessels. In sinuses reticular cells are more or less flattened to endothelial form and, with the sinal reticulum, which is here a basement membrane, comprise the wall of the vessels.‡ The cords have traditionally been considered an extravascular tissue. Here, the reticular cells which are thought to have the same developmental capacities as those lining the sinuses, have been described as stellate in form (Maximow & Bloom, 1957). Most of the phagocytosis and a great deal of cytopoiesis in the red pulp occur in the cords. In histological sections of the spleen, blood is usually present in the cords; and it is a question whether it is there by artefact or pumped in directly through open-ended terminal arterial branches and through apertures in the sinal walls. These alternatives represent, respectively, the concepts of the 'closed' and 'open' circulation of the red pulp of the spleen.

In a study of the structure of splenic sinuses in man and the rat (Weiss, 1957), the present author suggested that collapsed splenic sinuses constitute splenic cords. His conclusion was based primarily upon the observation that cords appeared lined by an endothelium identical in appearance to that lining the sinuses. Moreover, the endothelium of the cords lay base to base with the endothelium of the sinuses separated only by a common basement membrane, the sinal reticulum. As a result the sequence of structure in the red pulp was: sinal lumen, endothelium, reticulum, endothelium, lumen, endothelium, reticulum, endothelium, lumen. . . . The vascular character of cords was masked because their endothelium, highly irregular and often folded upon itself, would virtually obliterate the lumen.

These observations have been extended to the red pulp of the rabbit's spleen; and it has been concluded that, in rabbits, as in rats, and human being, the cords do constitute blood vessels. The endothelium of the cords obtruded upon the lumen mainly because it was phagocytic and cytopoietic. As a result, cord tissue appeared as extravascular spaces made up of irregularly stellate cells, and not as blood vessels. The purpose of this paper is to present these additional observations and the results

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‡ I have chosen the term *reticulum* among several accepted synonyms to refer to the delicate argyrophilic (or PAS positive) fibres described first by Mall (1896). Recently this reticulum, where it occurs beneath an endothelium, has been recognized as a basement membrane (Bennett, 1958). Hence I have referred to the latticed reticulum of the sinuses as the basement membrane. I have chosen the term *reticular cell* to refer to the relatively undifferentiated connective tissue cells associated with the reticulum, rather than the equally acceptable term *reticulum cell*.

of experiments designed to test them. In these experiments the red pulp was dilated in an attempt to convert cord tissue to frank vessels. Conversely, phagocytosis and cytopoiesis were forced upon the red pulp in an attempt to induce the endothelium of frank sinuses to lose its vascular character and make the vessels appear as extravascular cords.

The methods and observations will be presented in three parts. These are: (I) the red pulp of normal rabbits, rats, and human beings; (II) the red pulp of the spleen of rats and rabbits dilated by perfusion, by passive congestion alone, and by passive congestion in eserinated animals treated with acetylcholine or in animals treated with sodium nitrite; (III) the red pulp of rabbits and rats in which phagocytosis and cytopoiesis were induced by the haemolytic effects of phenylhydrazine.

Observations were made, primarily, with the light microscope upon splenic tissue fixed in buffered osmium tetroxide, embedded in methacrylate, sectioned at a thickness of approximately $2\ \mu$ in a Porter-Blum microtome, and stained with the periodic acid-Schiff reagents. This routine afforded well-fixed preparations of optimal thinness in which the basement membrane was selectively stained. The blocks of tissue, however, could not practically be more than a few mm. in greatest dimension. Conventional paraffin-embedded blocks of much larger size were also prepared. From the tissue embedded in methacrylate a limited number of electron micrographs were made.

I. THE RED PULP OF NORMAL RABBITS AND RATS

Materials and Methods

Young adult New Zealand rabbits weighing 2–3 kg., or young adult albino rats of the Wistar strain weighing 175–300 g., were placed under surgical anaesthesia with subcutaneous pentobarbital (60 mg./kg.). The spleen was removed, and portions were fixed in osmium tetroxide, dehydrated, and embedded in methacrylate according to the routine previously used (Weiss, 1957). In most cases no attempt was made to prevent blood from escaping the organ. In a few animals the vascular pedicle was clamped and the spleen fixed whole without loss of blood. After fixation the superficial few millimetres of the spleen were cut into small blocks, dehydrated and embedded by the usual routine.

Sections about $2\ \mu$ in thickness were cut from these blocks in a Porter-Blum microtome and mounted upon coverslips. The methacrylate was removed by xylene, and the tissue was stained with the periodic acid-Schiff-haematoxylin technique (PAS-H) as previously reported (Weiss, 1957). For paraffin embedding ethanol, formalin and glacial acetic acid (90:5:5) was used as a fixative.

Observations were made upon the spleens of 8 rabbits, 10 rats and 6 human beings.

Observations

Patent sinuses were prominent in the red pulp of the rabbit spleen. They were branching vessels of large but varying diameter consisting only of an endothelium and a fenestrated basement membrane. The cord tissue was slender, in many places but 5–10 cells thick (Pl. 2, figs. 2, 3; Pls. 3, 4).

By and large, the endothelium of the patent sinuses in an adult rabbit spleen was

low and seldom showed evidence of phagocytosis or cytopoiesis. The lumen of the sinuses contained blood, often with a higher concentration of granulocytes than peripheral blood. Moreover, in places, loaded-down macrophages were free in their lumen. The basal surface of the endothelium displayed varyingly prominent, but on the whole usually slight, longitudinal ribbing (Pl. 2, fig. 3). The basement membrane, stained with the periodic acid-Schiff method, was a prominent structure, and on surface view had the form of a grid (Pl. 2, fig. 2). Infrequently, the substance of the basement membrane was not confined to its grid pattern but appeared diffused into the lumen of sinuses or into cords.

The cords in the rabbit were slender structures of varied composition. In simplest form they consisted of facing cell surfaces separated by a slit or lumen of variable width. These cells appeared to form a cordal endothelium, and they lay upon the obverse side of the basement membrane of the adjacent sinus with the result that the basement membrane was common to sinus and cord. The cordal endothelium could be identical to the sinus endothelium. More often the cordal cells had more voluminous cytoplasm containing phagocytized material. Occasionally, the basement membrane was absent beneath pronouncedly phagocytic cordal cells, even where the endothelium of the adjacent sinus showed no phagocytosis. In occasional places in the cords, nests of plasma cells or megakaryocytes lay in the lumen or actually appeared to replace the endothelial cells. In the latter case, again, the basement membrane could be absent. Varying numbers of blood cells were present in the cords.

Similar observations were made in the white rat. Here, however, the cords were much broader than the sinuses. They were filled with blood and contained plasma cells, megakaryocytes, and occasional immature blood cells. Their endothelium was evidently actively phagocytic.

In human spleen, the transverse strands of basement membrane were thicker and more nearly at right angles to the longitudinal strands, and the sinuses were more branched than in rat or rabbit spleen. The cords resembled those of rabbit more than of rat (Pl. 2, figs. 4, 5).

Arterial terminations. The arterial endings were different from sinuses and capillaries in having a wall often two cell layers thick and in having a greater concentration of endothelial cells. In perhaps 20-25 % of cases in the rabbit a direct union of arterial ending and frank sinus was observed. Otherwise, the arterial ending opened into the lumen of what would be recognized as a cord. In the latter case, the squamous endothelium of the arterial ending was continuous with the irregular surfaces of the reticular cells of the cords. Often the arterial terminations bifurcated shortly before connecting with sinuses or cords (see Pl. 4).

II. DILATATION OF THE RED PULP OF THE SPLEEN IN RATS AND RABBITS

Materials and Methods

In rats of the Wistar strain and in New Zealand white rabbits, the spleen was engorged by ligating the splenic vein in animals placed under surgical anaesthesia with ether or subcutaneous pentobarbital. In some rats sodium nitrite was administered

intravenously a few minutes before ligation (Farris & Griffith, 1949). Others were eserized and then given 20 mg./kg. acetylcholine iodide intraperitoneally. The latter group shed bloody tears (Tashiro, Smith, Badger & Kezur, 1940). About 30 sec. to 1 min. after splenic ligation, the whole vascular pedicle was clamped and tied; and then the entire engorged organ was removed and fixed whole as suggested by Dr William Bloom. For paraffin embedding, Zenker's acetic acid mixture or ethanol, formalin, and glacial acetic acid (in the proportions 90:5:5) was used as a fixative. The latter was of practical use because it haemolysed the erythrocytes which otherwise were stained and obscured structures of greater interest. Tissue was fixed in osmium tetroxide and embedded in methacrylate as in Part I.

Serial sections were cut from the paraffin blocks and stained with periodic acid-Schiff reagents and haematoxylin. Occasionally, Bodian's silver stain (1936) or that recommended by Snook (1944) was used. From the methacrylate blocks thick sections were cut and stained with PAS-H.

The spleens of 5 rats and 3 rabbits were studied.

Observations (see Pl. 5)

The entire spleen was engorged with blood, although the degree of engorgement varied from place to place. In some areas cords or sinuses were compressed between widely dilated sinuses whose endothelium was markedly flattened. In most places what had presumably been cord tissue was filled with blood. In normal rat spleen this tissue often differed from frank sinuses in that reticular cells in an endothelial position were often filled with phagocytized material and were stellate rather than flat. Their voluminous cytoplasm protruded so deeply into the lumen that in many sections the cells appeared free. In many areas, however, there was a succession of congested vessels, undisguised by phagocytic endothelium (Pl. 5, fig. 16).

The movement of basement membrane from its netlike pattern into the sinuses and cords was marked in the engorged spleens (Pl. 5, fig. 16).

III. INDUCED PHAGOCYTOSIS AND CYTOPOIESIS

Materials and Methods

Normal rabbits and rats and argyric rats were treated with phenylhydrazine. The rabbits were young adult New Zealand animals weighing 2-3 kg. Each animal was given 48 mg. phenylhydrazine in saline, subcutaneously, each day for 3 days. Approximately 20 % of the animals failed to survive the week following the start of the experiment.

Normal Wistar rats and Wistar rats made argyric by the administration of AgNO_3 in drinking water (Gatz, 1949) for 12-24 months were given phenylhydrazine subcutaneously daily for 4 days as described by Smith & Stohlman (1934).

Observations were made upon the spleens of 21 rabbits, 10 normal rats, 10 argyric rats treated with phenylhydrazine, and 3 rats treated with AgNO_3 alone.

*Rabbit**Observations*

Following the start of phenylhydrazine more or less normal relationships of sinuses and cords persisted a few days. Then the spleen became congested. After this the sinal endothelium became phagocytic, the basement membrane disappeared, and the sinuses became indistinguishable from cords whose reticular cells had become more markedly phagocytic. Cords merged with sinuses; and as phagocytosis became more pronounced extensive stretches of red pulp were filled with huge, closely packed, swollen phagocytes, with no sensible vessels visible. The structure of the red pulp was massively deranged.

On the fourth day of the experiment, when the circulating haemoglobin was typically about 5 g. % and the reticulocytes 25–30 %, the frank sinuses and cord tissue were widely congested, even more uniformly and more pronouncedly than could commonly be attained by splenic vein ligation and arterial dilatation (Pl. 6, figs. 24, 25). The basement membrane was well stained. In the next day or two, congestion remained extreme and phagocytosis was only of moderate degree. But the endothelial cells and the basement membrane of sinuses were altered; huge, odd-shaped, vividly stained phagocytes lay upon the basement membranes and free in the masses of erythrocytes, and the continuity of the basement membrane was disrupted in many places (Pl. 6, fig. 27; pl. 7, figs. 28–32). On the sixth or seventh day phagocytosis was more pronounced in both the cordal and sinal endothelium, with the changes in the cords in advance of those in the sinuses. Two events associated with phagocytosis—the loss of material from the basement membrane, and of the squamous character of the endothelium in sinuses—made it impossible to distinguish cord from sinus in many places in the spleen.

At this stage huge mononuclear cells loaded down with broken red cells were crowded in the lumen of many sinuses and cords. In about 10 days the lumina of those frank sinuses which remained were relatively free of these phagocytes, while the now expanded cords were packed with them. The topography of the red pulp had become considerably changed from normal. The tissue was still congested, cords were greatly expanded, and fewer sinuses were present, having been absorbed into the broadened cords. Many of the sinuses had become irregular blood-filled clefts unlined by endothelium (which had presumably been swept away after becoming phagocytic) and unmarked by basement membrane, in a mass of huge, closely packed, swollen phagocytes.

It must be emphasized that the broadening of the cords was not due to expansion of existing cords with compression and apparent disappearance of sinuses lying between the cords. Nor was it due to stuffing of a sinus with sequestered phagocytes so that it appeared cordal. Rather, the sinuses lost their vascular character because their endothelium became phagocytic and their basement membrane disappeared. They then became indistinguishable from cord tissue and merged with pre-existing cords.

The larger sinuses, similar to those free of blood in Pl. 1, fig. 1, which became confluent with splenic veins, were unresponsive to phenylhydrazine. The changes described above were characteristic of smaller sinuses.

The arterial terminations in phenylhydrazine-treated rabbits were noteworthy for clusters of macrophages at the orifice of the ending. Not uncommonly, the endothelium of one portion of a sinal wall near which an artery ended had become phagocytic and irregular while the rest of the wall stayed flat (Pl. 8, fig. 37).

Rat

Both congestion and phagocytosis in the spleen followed a course of phenylhydrazine in the normal rat. As with the rabbit, phagocytosis occurred first in the cords but, possibly because the cords in rats are more capacious, seldom extended to the sinal endothelium even with very large doses of phenylhydrazine.

In silver nitrate-treated animals, on the other hand, several of the effects similar to those obtained with phenylhydrazine resulted from the administration of silver alone (Pl. 1, fig. 1). Many macrophages were filled, presumably with silver, and the reticulum, particularly in and somewhat beyond the marginal zone, was blackened. The cords had become more cellular, and in some silver-treated animals the number of recognizable sinuses was actually reduced. In these animals the conversion of what has presumably been frank sinal tissue to cords after phenylhydrazine was more nearly complete than in any other group (see Pl. 10). Typically, several high-power fields in succession consisted almost entirely of closely packed macrophages and blood without any sensible sinuses. The non-sinal tissue had the appearance of a meshwork of phagocytic reticular cells and blood. In addition, the endothelium of several sinuses had become phagocytic and, only by means of some persistent basement membrane and its luminal contour, could its sinal character be inferred.

Few infarcted areas were present either in rabbits or rats treated with phenylhydrazine.

DISCUSSION

The major interpretations of the structure of the vascular bed of the red pulp have been set within the conception of sinal vessels separated by extravascular cords (Björkman, 1947). The presence of blood in these cords has required the theory that blood may normally flow extravascularly or the belief that histological techniques have resulted in the breaking of sinal walls with artefactual extravasation of blood. The interpretation, presented here, of cords as blood vessels having a responsive endothelium whose activities may mask their vascular nature emphasizes the reactivity and potency of the reticuloendothelial system and harmonizes heretofore conflicting observations without requiring the singular theory of extravascular flow or the conclusion that histological technique produces major artefact.

The technique of fixation in osmium tetroxide, embedding in methacrylate, sectioning at 2μ or less, and staining with periodic acid-Schiff and haematoxylin affords sections of optimal thinness, excellently fixed with little shrinkage—in many respects superior even to celloidin embedding—in which the basement membrane is selectively stained and the cells well rendered. The blocks may also be sectioned for electron microscopy.

Interestingly, by his injection methods, Lewis (1957) concluded that sinuses are but minor modifications of pulp spaces. Indeed a close relationship of cord to sinus

was established by Mollier (1911) and Koboth (1939). In the rabbit cordal vessels appear in the present work to be sinuses, but masked by the activity of their endothelium. In the rat and human spleens, however, cordal vessels appear somewhat different from simply masked sinuses. In the rat the cords are broader. In the human being the cords are slender, and the cordal reticulum may form a mantle about the sinus as described by Koboth (1939). Conversely, Snook (1950) has emphasized species variations in the structure of sinuses.

While reticular cells must be recognized as relatively undifferentiated cells, there is in these experiments no evidence that they readily undergo differentiation into erythroblasts in the spleen. In fact, only a few would appear to undergo this transformation, perhaps more a reflexion of the power of the bone marrow than on the limits of the spleen. Most erythroblasts in the spleen have been sequestered as indicated by their presence in the spleen only when they circulate in the blood, and the absence in the spleen of any forms less mature than in the blood.

Even the capacity of reticular cells to become phagocytic would appear to vary from place to place in the red pulp. The larger sinuses and those in union with veins, though made of endothelium morphologically similar to that of tributary sinuses, remained unmistakably patent vessels. The endothelium of the tributaries was phagocytic. Whether the endothelium of the larger vessels was intrinsically less responsive or whether the blood was cleared of damaged red blood cells before reaching their endothelium is not known. Biozzi, Halpern, Benacerraf & Stiffel (1957) demonstrated that the reticuloendothelial system may be stimulated to enhanced phagocytosis by preliminary phagocytosis of injected particles, and suggested that this may be due to new reticular cells produced by mitosis. I have noted greatly increased frequencies of mitosis among the reticular cells in sites where phagocytosis is marked.

Under experimental conditions, as in the undisturbed spleen, phagocytosis occurs preferentially in the cords. Several factors may enhance phagocytosis in an already phagocytic endothelium: viz. the surface of a phagocytic endothelium is irregular and would presumably slow down blood flow; more arterial terminations end in cordal vessels than in sinusal vessels; the possible enhancement of phagocytosis by new cells has been noted in the previous paragraph; in places the basement membrane is washed into the paludal spaces of the cords where it coats red cells and may facilitate their ingestion.

Motulsky, Casserl, Giblett, Broun & Finch (1958) have reported that blood, tagged with radioactive ^{51}Cr , normally flows rapidly through the spleen as through other organs. Therefore, in all likelihood it normally travels through frank vessels. For although the proportion of arterial terminations communicating with frank vessels is small, these vessels would appear able to convey most of the blood flow in the spleen because the number of fine vessels normally open in any capillary bed at any time is small and flow through frank vessels is efficient. Moreover, the observation that Kupfer cells remove most carbon and other particulate material given intravenously in moderate dosage, although the phagocytic prowess of the spleen is demonstrably greater (Biozzi *et al.* 1957), support the more direct measures (Motulsky *et al.* 1958) that the volume of the blood flow through the normal spleen is relatively low.

In abnormal spleens (for example those reacting to methylcellulose, in haemolytic anaemias, and presumably in the haemolytic anaemia after phenylhydrazine) the volume of blood is increased and blood flow is greatly slowed down (Motulsky *et al.* 1958). Here, in all probability, most of the blood flows through cordal vessels whose structure, as discussed above, prevents rapid flow and gives these vessels the deceptive appearance of extravascular tissue.

Lying between the endothelium of vessels made only of endothelium in a tissue made entirely of vessels, the basement membrane represents a continuous branching complex of fenestrated surfaces by which the disposition of sinuses and cords can be inferred. The fenestrations in the basement membrane are large, leaving a slender netted structure made up of regularly spaced strands. The opening in the basement membrane would appear to allow the endothelium of one vessel to present on the lumen of its neighbouring vessel, if that vessel's endothelium had been lost as a detached mononuclear blood cell, megakaryocyte or loosened phagocyte. Lymphatic capillaries in the rat diaphragm lack a basement membrane, and the abluminal surface of the endothelium may become actively engaged in pinocytosis, and transport great quantities of thorotrast or other materials into the lymphatic capillary from the extravascular spaces (Fraley & Weiss, 1959). Perhaps the absence of basement membrane from what is actually the greater part of the abluminal surface of the sinal endothelium permits such active transport across the sinal wall. The red pulp of the spleen, indeed, as in the case with other tissues provided with sinal blood vessels, contains no lymphatic vessels; their functions here may be subserved by the sinuses. With regard to the control of blood flow, the remarkable rectilinearity of the basement membrane suggests that this structure may constitute a grid or set of coordinates upon which the endothelium is aligned. Since the basement membrane may disappear as its overlying endothelium becomes phagocytic, self-regulation of blood flow may be inherent in the structure of the sinuses. For as the endothelium becomes phagocytic and moves from the wall and the basement membrane disappears in this system of vessels sharing common walls, a new entrance to the adjoining vessel is blocked, if at all, only by a motile unsupported endothelial cell. Since the basement membrane may be secreted by the endothelium and depends upon the endothelium for maintenance, all of the factors required for recasting their vessels appear to lie in the vessels themselves. In the process sinal vessels by reason of their responsive endothelium lose their frank vascular appearance and become cords of tissue having no resemblance to blood vessels and every characteristic of extravascular tissue except the presence of blood. Under appropriate conditions the transformed endothelium may be swept out (to be sequestered in other cordal tissue or liver or lung in the case of macrophages, or to the circulation in the case of lymphocytes or monocytes), the wall re-covered with new endothelium and the basement membrane restored. Perhaps the layout of sinuses and cords is worked and reworked in a manner similar to the Haversian systems of bone, responsive in this case to the requirements for phagocytosis, antibody production, platelet production, metaplastic cytopoiesis, sequestration of blood cells, and blood flow.

SUMMARY

Reticular cells in the red pulp of the spleen are organized as sinuses, and as cords separating the sinuses.

Splenic cords are here interpreted as unstable, responsive blood vessels whose vascular nature may be disguised by collapse of the vessels with apposition of the irregular endothelium and virtual disappearance of the lumen, and by phagocytosis and cytopoiesis in the endothelium with resultant loss in endothelial form. The endothelium of the sinuses is on the reverse surface of the basement membrane of the cordal endothelium. Thus the repeating sequence of structure in the red pulp is: endothelium, lumen, endothelium, basement membrane, endothelium, lumen....

Arterial terminations may empty in sinal or cordal vessels but many more empty into cords.

Cordal vessels may be experimentally dilated by congestion of the spleen. Their vascular nature is then revealed because collapsed vessels are opened and phagocytic endothelium tends to be pressed back into endothelial position. Sinuses may be converted to cordal tissue by forcing phagocytosis upon their endothelium by the haemolytic effects of phenylhydrazine.

The basement membrane appears to depend upon the endothelium for maintenance, and under certain conditions it may disappear or its substance appear to wash into the lumen of sinus or cord.

Thus the red pulp is almost entirely a vascular space whose vessels are unmistakably vascular, or masked as they carry out the functions of the spleen; viz. phagocytosis, cytopoiesis, sequestration of cells. As a result of the disposition of reticular cells into a responsive system consisting only of endothelium and basement membrane control of these reticuloendothelial functions would appear vested in the reticuloendothelial system itself.

I wish to acknowledge the technical services of Miss Adele Barbeau who made the histological preparations and Mr Leo Talbert who made the photomicrographs.

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EXPLANATION OF PLATES

PLATE 1. Argyric rat spleen

A nodule of white pulp is surrounded by red pulp. The reticulum, including basement membranes, is vitally stained with silver and also stained with the PAS reaction. Macrophages, concentrated in the marginal zone and present elsewhere, are also doubly stained. Note the tendency to circumferential disposition off the sinuses in the red pulp just outside the marginal zone. From these vessels, branches spring in a radial direction. The netted appearance of the sinal basement membrane is rendered prominent by the silver. At the bottom of the photograph, a pulp vein enters a trabecular vein.

The tissue was fixed in alcohol-formalin acetic acid (90:5:5), embedded in paraffin, stained with PAS, and photographed $\times 300$.

PLATE 2. Normal rabbit and human red pulp

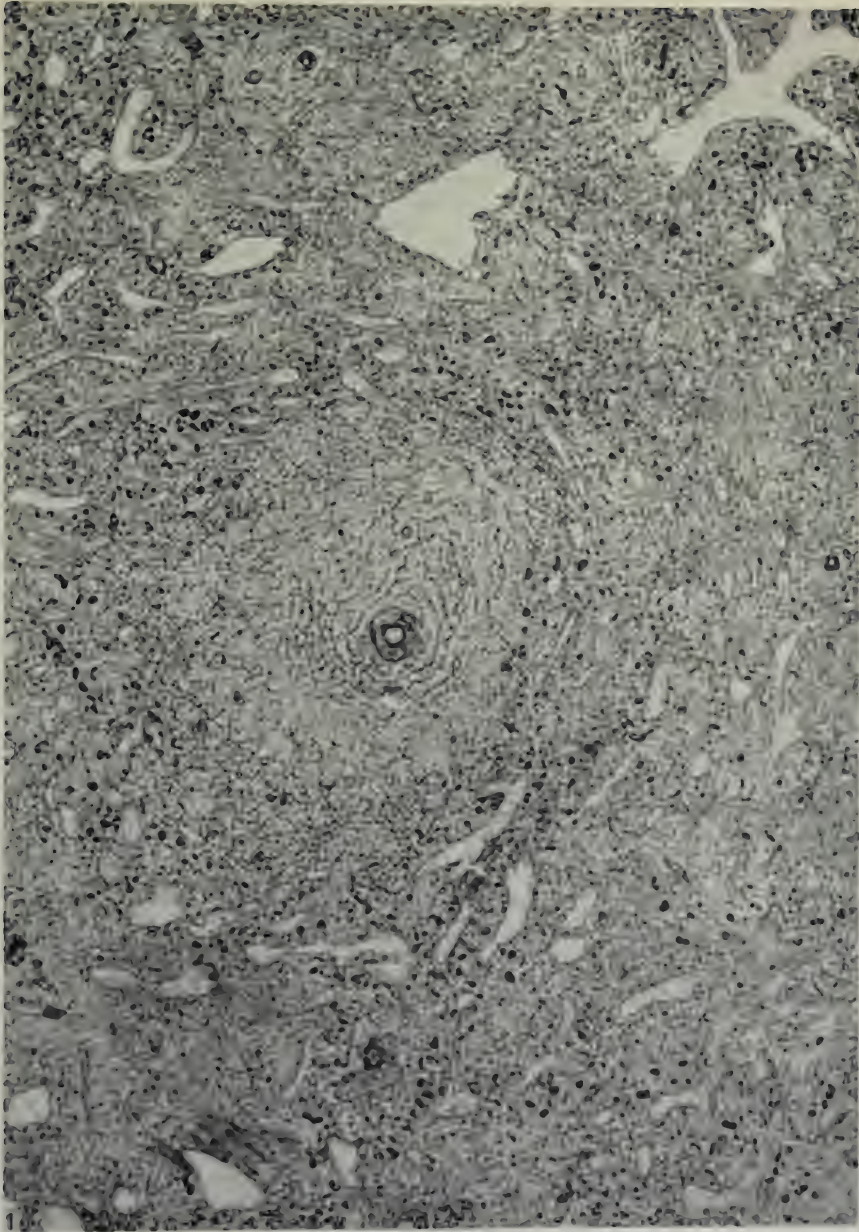
Figs. 2, 3. Rabbit red pulp. These photographs are of the same field at different levels of focus. A sinus crosses the field from left to right. On the right side of each photograph, the section grazes the sinus's wall; and on the left it passes into its lumen. In fig. 2, on the right, the fenestrated basement membrane, selectively stained with the PAS technique, is in focus. In fig. 3 the basal endothelial surface is in focus. Note the endothelial nuclei and the more or less longitudinal orientation of the endothelial cytoplasm.

This vessel branches from its lower margin. Part of the basement membrane (see particularly fig. 2) and endothelial nuclei of this branch may be seen in figs. 2 and 3. Note a portion of lumen of this branch at the arrow in fig. 3. Its endothelium is phagocytic, as may be seen to advantage at the arrows in fig. 2.

At the upper right corner of each photograph is a portion of a frank sinus. Between it and the vessel crossing the field from left to right is cord tissue whose vascular nature is masked. Figs. 4, 5. Human spleen. The sinuses branch and communicate, outlined by the selectively stained basement membrane. Fine basal endothelial striations, more highly developed than in the rabbit, are marked by arrows in transverse sections of a sinus or where the section cuts the basal surface of an endothelium. In fig. 5 the portions of sinus, each labelled *a*, are continuous with one another.

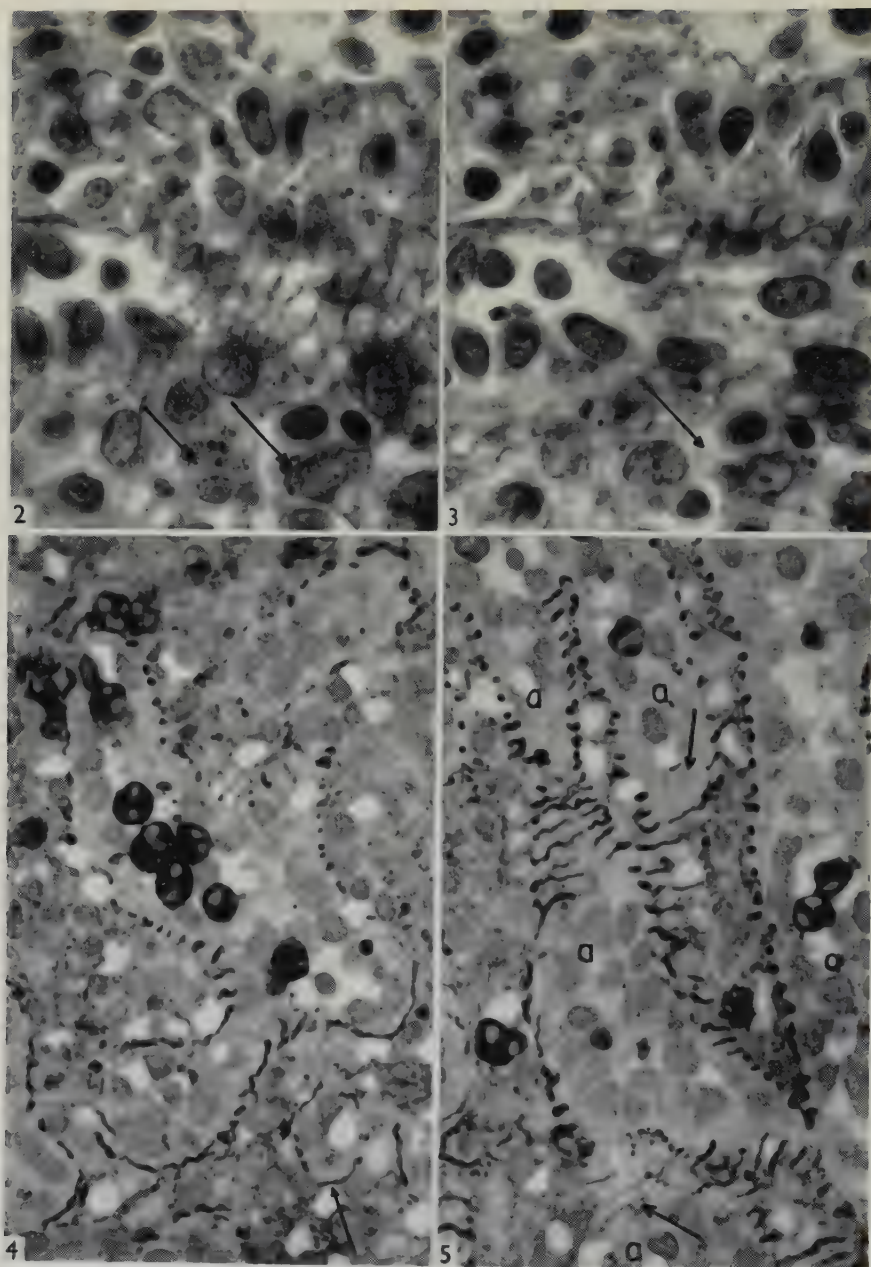
The deeply stained spherical cells in the sinal lumen are neutrophils rich in glycogen.

The spleen was fixed in osmium tetroxide, embedded in methacrylate, sectioned at about $2\ \mu$, stained with periodic acid-Schiff and haematoxylin, and photographed $\times 1100$.

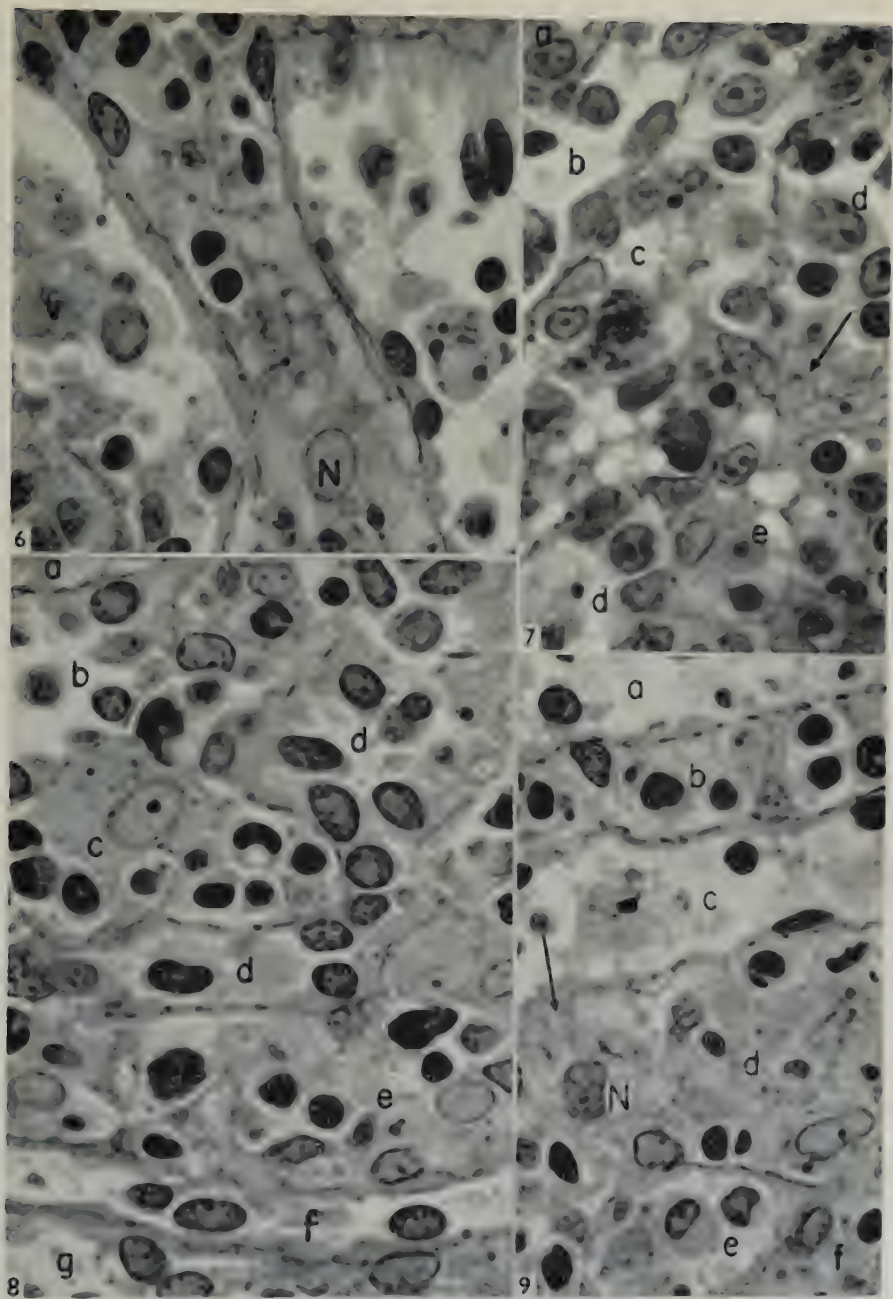


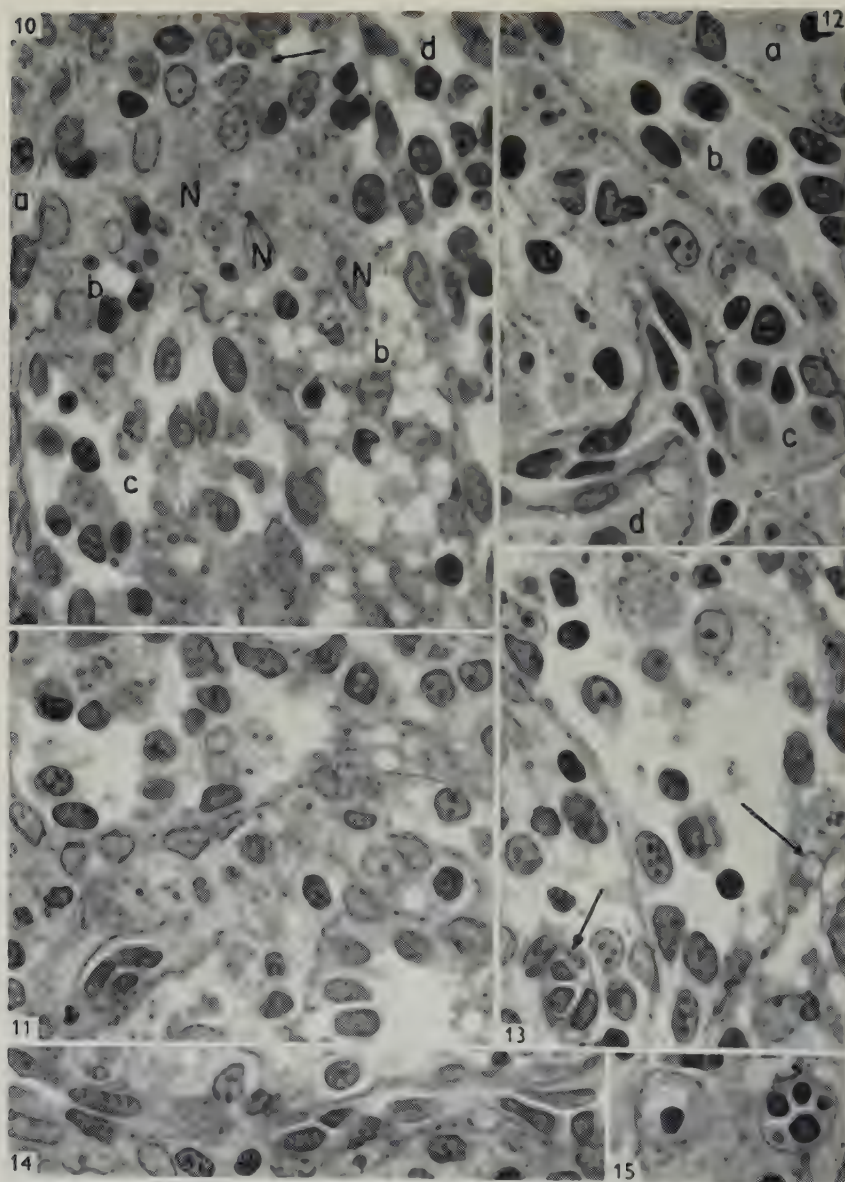
WEISS—RED PULP OF SPLEEN

(Facing p. 474)

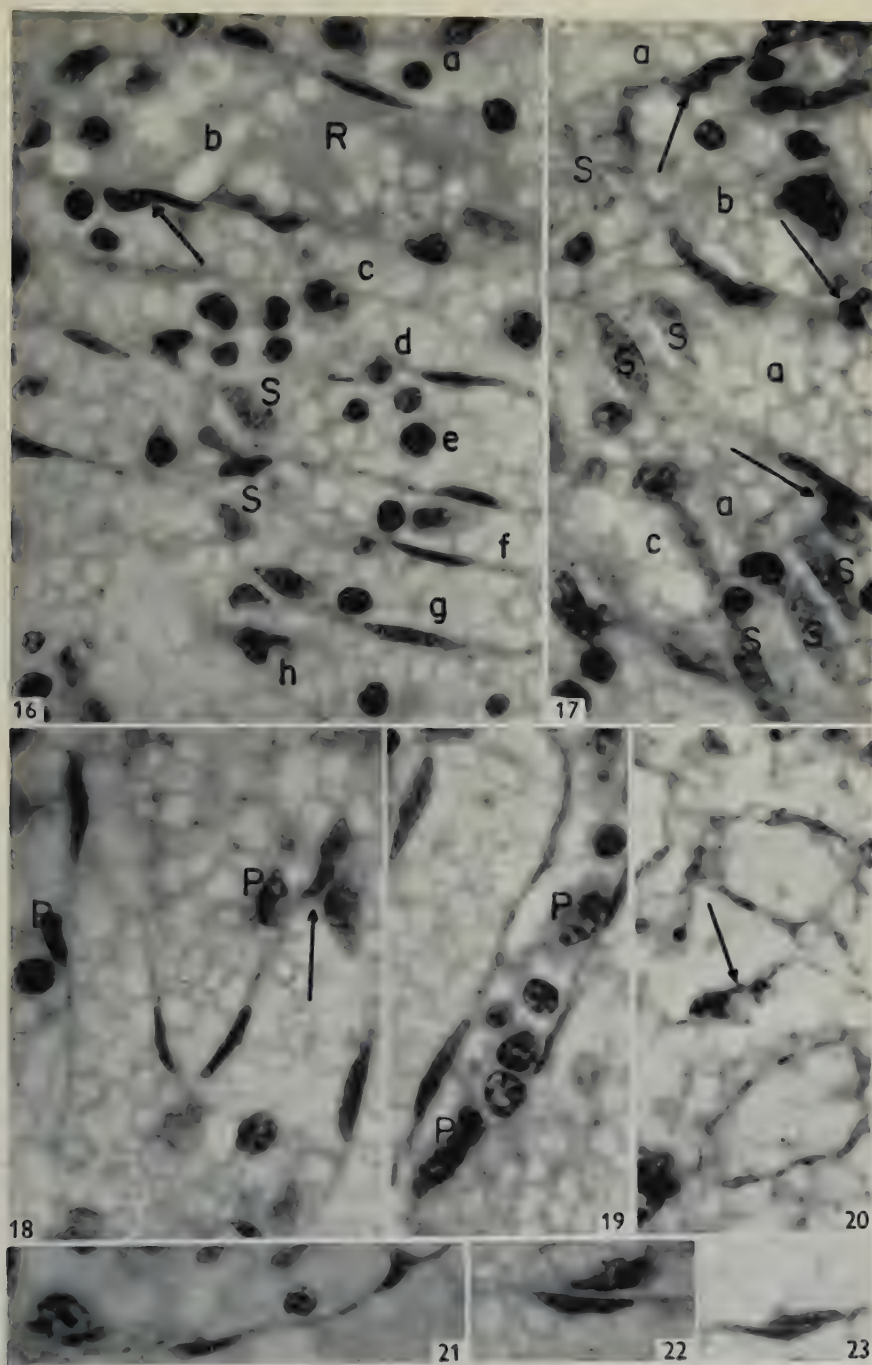


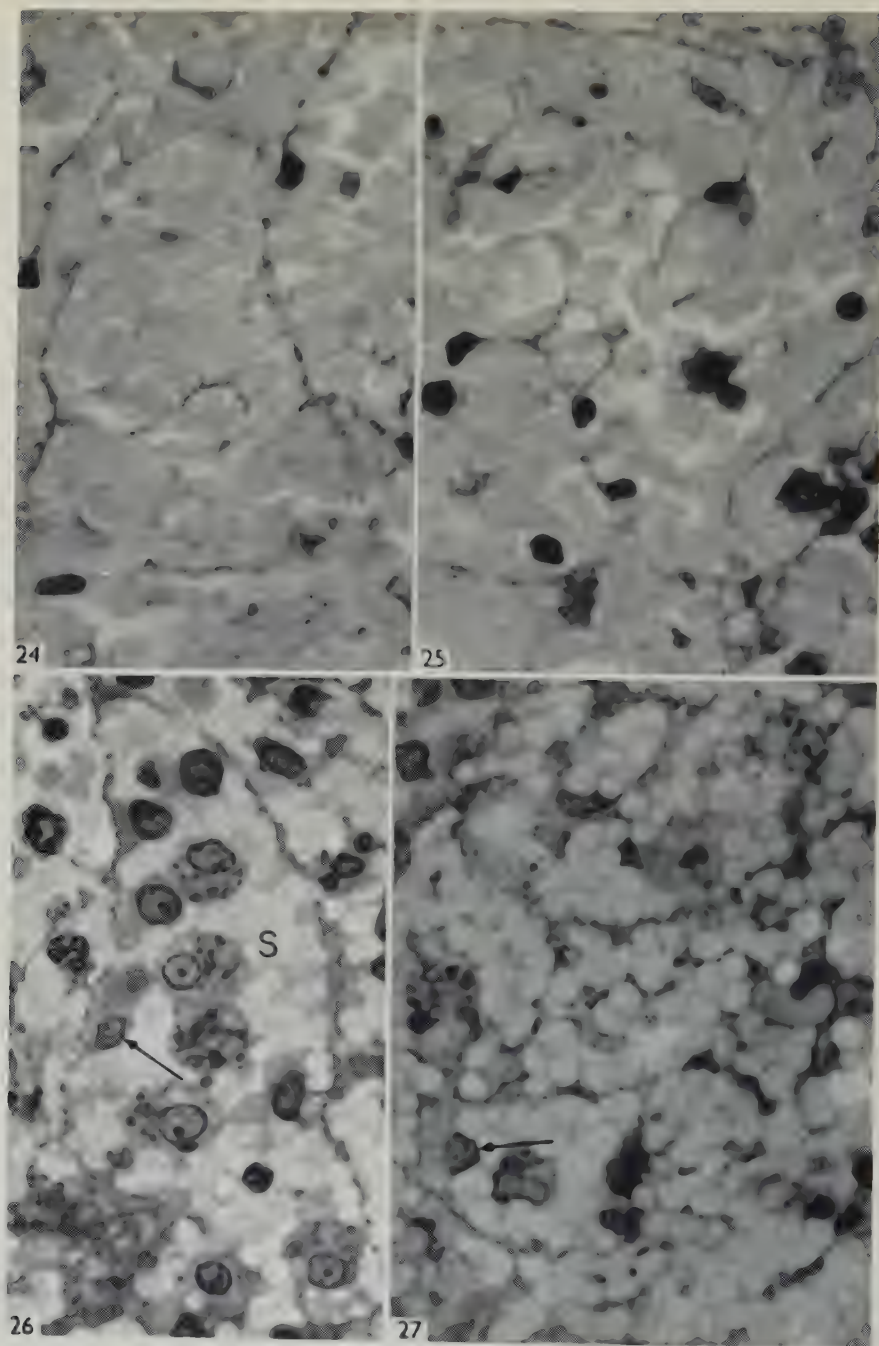
WEISS—RED PULP OF SPLEEN



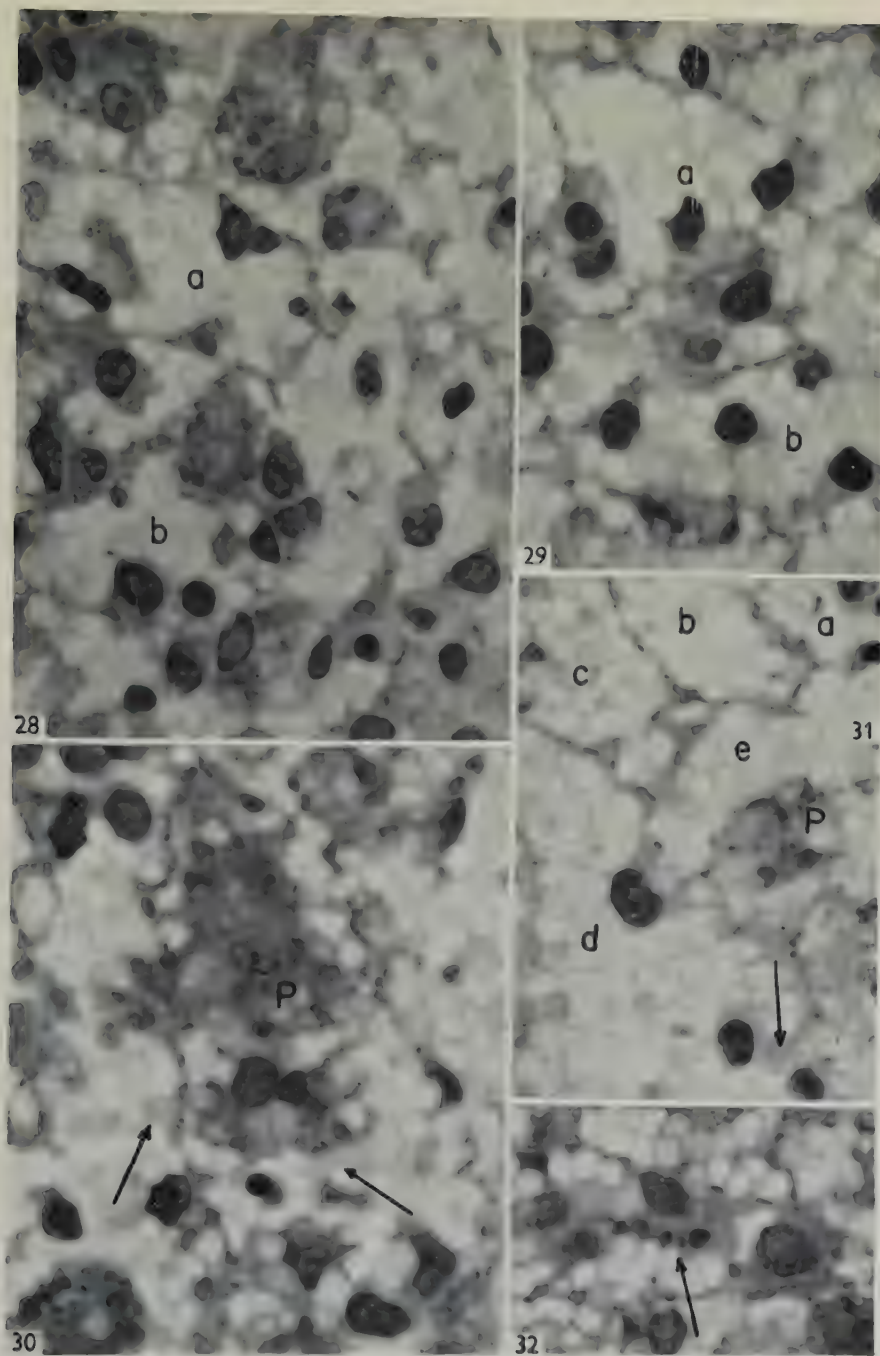


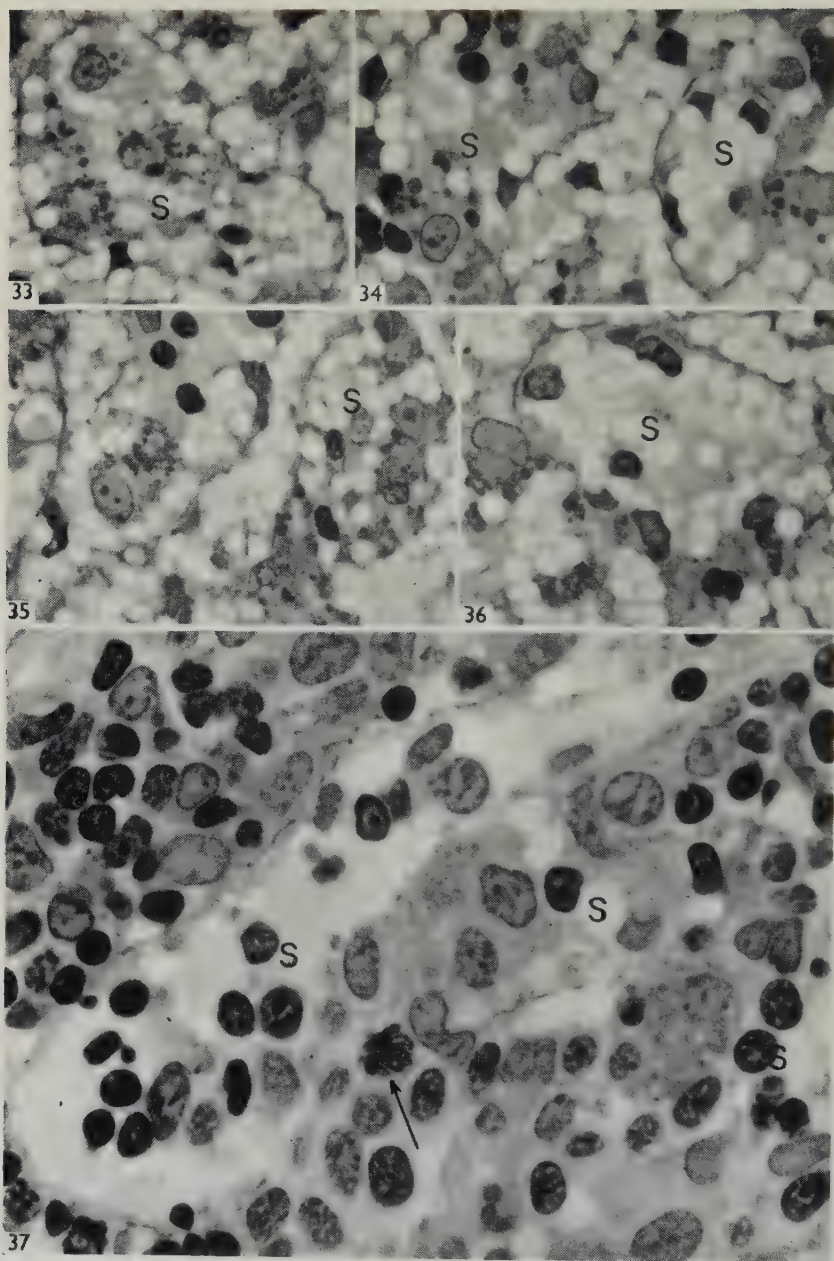
WEISS—RED PULP OF SPLEEN

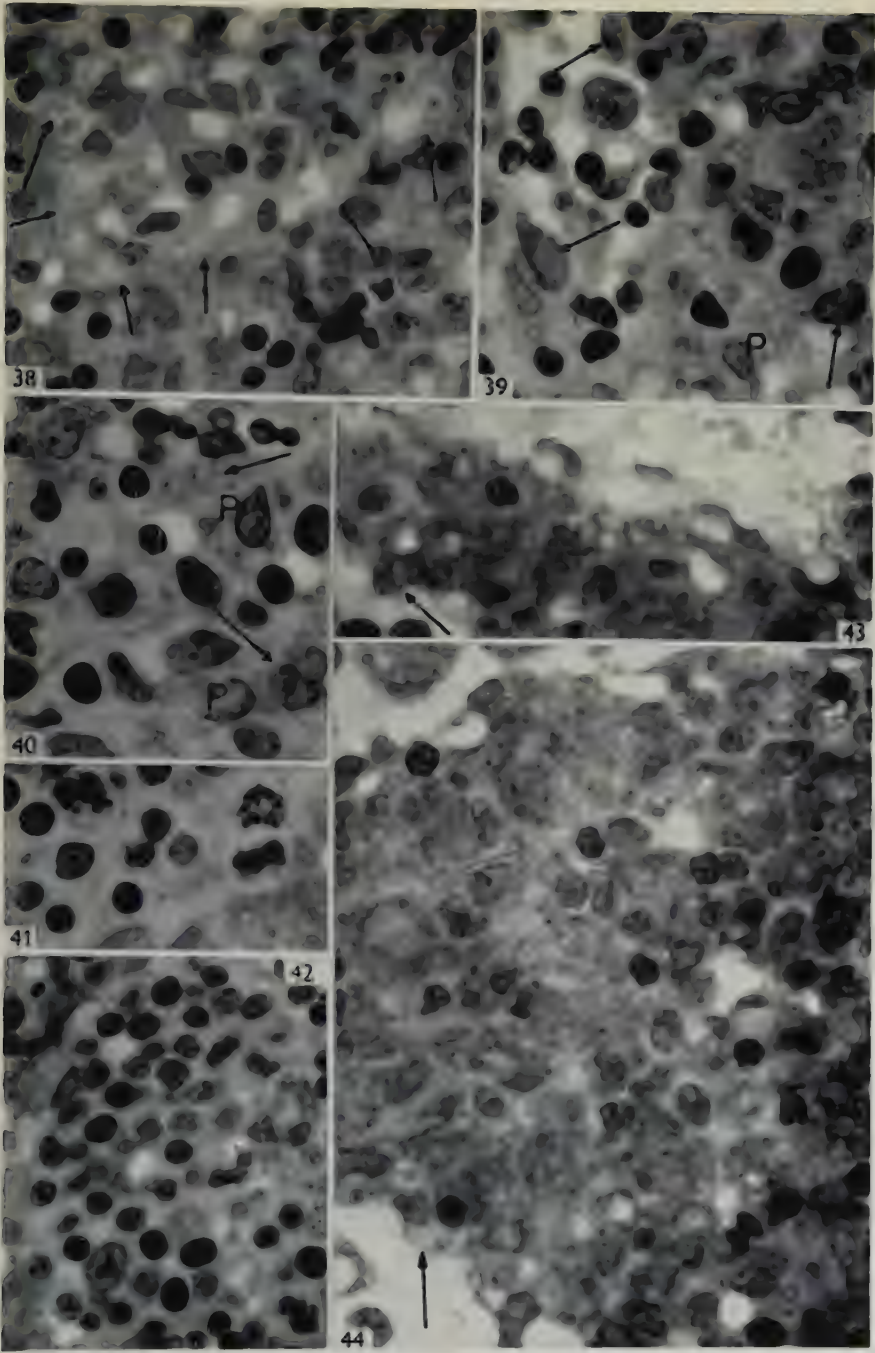




WEISS—RED PULP OF SPLEEN







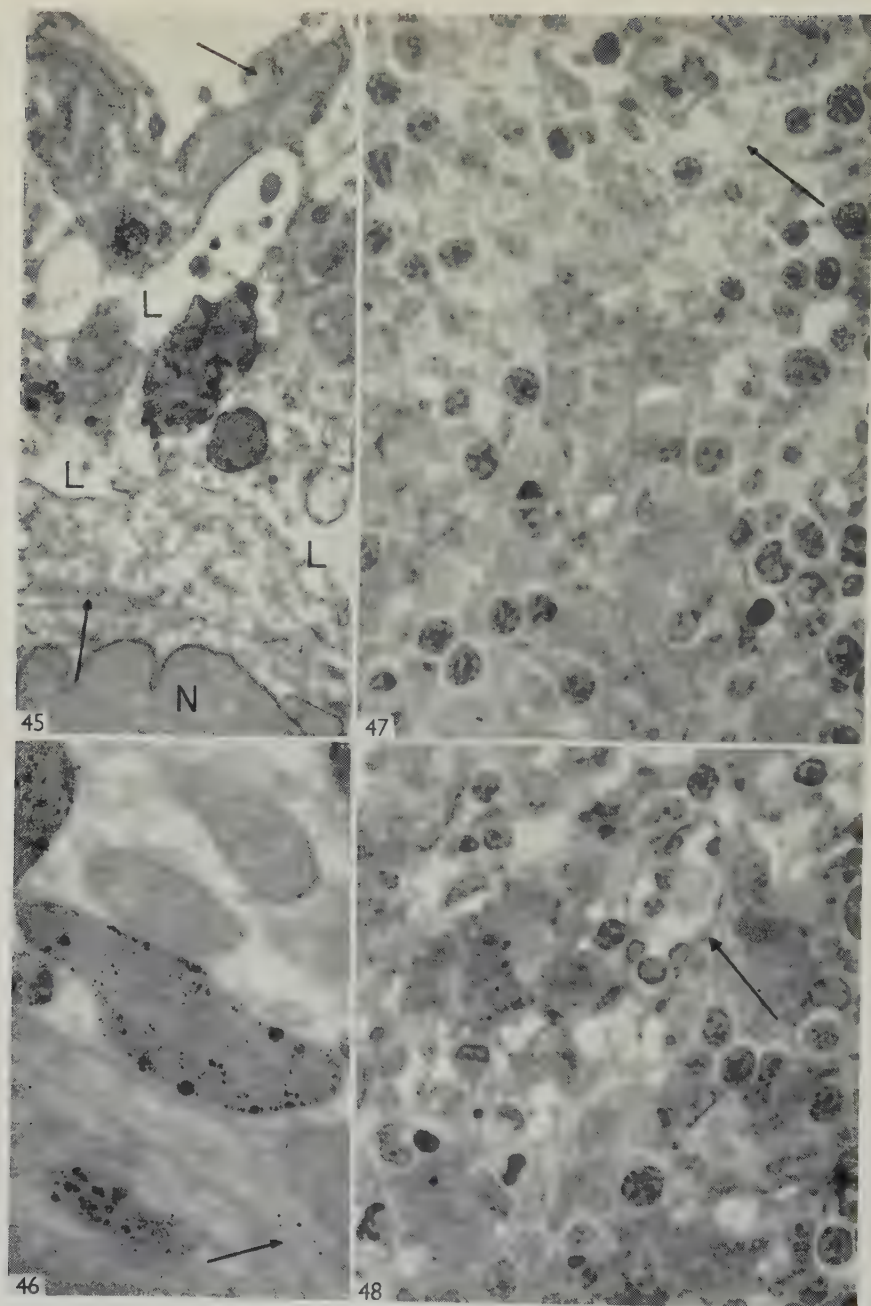


PLATE 3

These photographs of normal rabbit red pulp illustrate relationships of sinuses to cords.

In fig. 6 a cord separates two frank sinuses which contain macrophages. Three endothelial nuclei are present in the cord; the lower one, cut parallel to the cordal surface, is phagocytic. It is labelled 'N'.

Fig. 7 is a portion of the red pulp which may be analysed as a succession of vessels, indicated *a, b, c, d, e*, varying in endothelial reactivity, tortuosity, and luminal content, but alike in having an endothelium lying upon a basement membrane. The letter 'a' touches an endothelial nucleus of a vessel largely out of the field. The letter 'b' is directly over an endothelial nucleus and to the left and slightly below 'c' is an endothelial nucleus. Similarly, an endothelial nucleus lies to the right of the lower letter 'd'. Note that vessel *d*, running upward and to the right from the lower left corner narrows, is cut tangentially, and then, as the section passes again into the lumen, expands. An arrow marks the tangential cut and here the basement membrane may be seen on surface view.

In fig. 8, again, the field may be interpreted as a succession of vessels sharing common walls. The lumina are designated *a, b, c, d, e, f, g*. Vessels *d* and *c* have a highly irregular contour.

The field in fig. 9 contains three frank sinuses marked *a, c* and *e*, the latter oval in outline. The structures designated *b* and *d* are cords. Despite phagocytosis in *d* and possibly sequestration of cells in *b*, their vascular nature may be recognized by their configuration and the presence of endothelium. Note the surface view of the basement membrane in cord *d* at the arrow. As the section is followed in the direction of the arrow, it passes closer to the lumen, exposing an endothelial nucleus (N).

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ , stained with PAS-H, and photographed $\times 1100$.

PLATE 4. Normal rabbit red pulp

Examples of terminal arterial endings are present.

Some vessels designated *a, b, c* and *d* are present in fig. 10. *c* and *d* are frank vessels, *c* containing many macrophages. *a* is barely seen in the photograph. Vessel *b* branches, one limb to the right, clearly vascular, the other to the left, mostly out of the field. At the bifurcation, the section passes through the wall of the vessel, exposing endothelial nuclei (N) and delicate strands of basement membrane. This endothelium appears phagocytic. At the upper margin an arterial termination is present in cross section (arrow).

In fig. 11 frank vessels along the upper margin and at the right lower corner of the photograph are separated by a cord whose vascular nature may be inferred by the presence of endothelium. Marked phagocytosis is evident here. An arterial termination appears to empty into the cordal tissue.

Fig. 12 illustrates a common type of arterial ending. The terminal vessel bifurcates to form a T-shaped structure which ends in a cordal vessel. Again, note the succession of vessels *a, b, c* and *d*.

In fig. 13 an arterial termination ends in a frank sinus. Along the right margin of the sinus (arrow), the section verges to the tangential exposing the netted pattern of the basement membrane. Below and to the left (arrow), a terminal arterial vessel is cut in cross-section. Compare with fig. 7 of Björkman's work (1947).

In figs. 14 and 15 the terminal arterial segments cut in longitudinal and cross-section. The vessel dips in and out of the field in fig. 14. Note the difference in number of nuclei in the two segments of fig. 15.

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2 μ , stained with PAS-H, and photographed $\times 1100$.

PLATE 5

The photographs are of rat spleen in which the splenic vein was tied off after the administration of sodium nitrite. The whole spleen was fixed in alcohol, formalin, acetic acid, embedded in paraffin, sectioned, stained with periodic acid-Schiff and haematoxylin, and photographed at a magnification of 1100.

The red pulp is markedly congested, the red blood cells unstained. Many nuclei are unusually flattened by the increased venous pressure. Compare these photographs with figs. 24–25.

In fig. 16 there appear to be eight vessels (*a-h*) running approximately parallel to one another. Note at the arrow the endothelial nuclei, back to back, separated by a basement membrane. (Additional examples are present in figs. 22 and 23. In fig. 23 one of the nuclei, presumably that of a dilated cord, is phagocytic.) The nuclei marked 'S' are endothelial nuclei on surface view. At 'R' the lumen is filled with what appears to be the substance of the basement membrane or reticulum washed from its basal location into the lumen.

In fig. 17, several limbs of vessel *a* diverge from the region marked by the topmost 'S'. In several places the cut is tangential and the fenestrated basement membrane shown. Note the surface aspect of the endothelial nuclei at these places (*S*). At the lowermost arrow, the section includes a profile of a phagocytic endothelial cell which lines the lower limb of vessel *a* together with the tangentially cut cells marked *S*. A flattened endothelial nucleus, of the middle limb of *a*, lies on the other side of the basement membrane. Vessel *b* is presumably a dilated cord. Several of its cells are endothelial in position (arrows) in this section. Vessel *c* appears to be cut in oblique section.

A bifurcating vessel is present in fig. 18. Its right limb tapers to a point at the arrow. Phagocytic endothelial cells of the cordal vessels are labelled *P*. The cordal vessel in fig. 19 is compressed. It contains two phagocytic endothelial cells (*P*). Fig. 20 lacks the nuclear counterstain. The basement membrane is stained with the periodic acid-Schiff reaction. Again, note the succession of engorged vessels. At the arrow the contour of a vessel is completed by an endothelial cell whose phagocytized contents are stained.

A segment of basement membrane is present in fig. 21. A flattened endothelial nucleus is present on the lower surface and two endothelial cells (the one on the left obviously phagocytic) present on the obverse surface. In figs. 22 and 23 endothelial nuclei are present on obverse and reverse surfaces of basement membrane.

PLATE 6. The red pulp after administration of phenylhydrazine—early changes

The tissue in figs. 24 and 25 was fixed in Orth's fluid, embedded in paraffin, and stained with PAS-H. The red pulp is markedly congested with red cells, dilating cordal and sinal tissue. Surprisingly, few endothelial cells are present in fig. 24. Perhaps some of the endothelium came off the wall and was swept out. Compare with Pl. 5.

In figs. 26 and 27, normal sinal structure is disrupted. Note the endothelium in the sinus (*S*) of fig. 26 protruded deeply into the lumen. Several of the endothelial cells are phagocytic (arrow), and some free phagocytes are present in the lumen. At places the sinal wall is broken. Most of the erythrocytes are unstained, but several are coated with what appears to be the substance of the basement membrane. See also Pl. 7. In places this material is free in the plasma. In fig. 27 note the phagocytic sinal endothelial cell at the arrow.

The tissue in figs. 26 and 27 has been fixed in osmium tetroxide, embedded in methacrylate, sectioned at approximately 2μ , stained with PAS-H, and photographed $\times 1100$.

PLATE 7. The red pulp after administration of phenylhydrazine—early changes

The pulp is congested, almost all red cells unstained. Endothelial cells protrude from the walls of sinuses, the continuity of the walls is often broken, and in places the basement membrane has been washed into the plasma (for example, fig. 31, arrow) and on to occasional red cells (for example, fig. 30, arrows).

Disrupted sinuses may still be recognized at *a* and *b* in fig. 28; *a* and *b* in fig. 29; *a*, *b*, *c*, *d* and *e* in fig. 31. Note the endothelial cells which have become phagocytic, for example those labelled *P* in figs. 30 and 31 and at the arrow in fig. 32.

This tissue has been fixed in osmium tetroxide, embedded in methacrylate, sectioned at approximately 2μ , and stained with PAS-H, and photographed $\times 1100$.

PLATE 8. The red pulp after administration of phenylhydrazine—later changes

Many endothelial cells retain their attachment to the basement membrane, but their cytoplasm protrudes irregularly into the sinal lumen (*S*) and is loaded down with fragmented and whole red cells. By and large, the basement membrane persists, but in many places it is absent.

In fig. 37 an arterial termination (largely out of the field) enters a sinus at the right upper corner. The sinus (*S*) may be followed to the right and then down where part of its endothelium appears to have become phagocytic, although some of the phagocytes may have come away from the walls of other sinuses and been trapped here. Note the cell in mitosis (arrow) and to its right the constricted nucleus of cell, apparently squeezing through a narrow aperture in a sinal wall. Compare with fig. 7 in a previous paper (Weiss, 1957).

This tissue has been fixed in osmium tetroxide, embedded in methacrylate, and stained with PAS-H. Figs. 33-36, $\times 1100$; fig. 37, $\times 1350$.

PLATE 9. The red pulp after administration of phenylhydrazine—later changes

In fig. 38, endothelium of sinus is voluminous and occludes the lumen, rendering the limits of the sinus vague except where basement membrane (arrows) persists.

In fig. 39, a sinus runs from the left upper corner toward the right lower corner and then up. Several endothelial nuclei may be recognized (arrows). The basement membrane is present along most of the vessel's course but is not prominent. At one place a phagocytic endothelium (*P*) blocks the lumen. The arrows and the letter *P* lie in the lumen of one vessel.

Another sinuous vessel may be followed in fig. 40. Its basement membrane is indicated by arrows at two points. At the upper arrow the section grazes the surface of the basement membrane. Directly across the basement membrane from the lower arrow lie two nuclei of the endothelium of the adjoining sinus. Two phagocytic endothelial cells are marked '*P*'. The arrows and letters lie within the lumen of one sinus.

The number of mitoses, illustrated in figs. 41 and 42, in this material is considerably greater than normal. Note the sinus occupying most of the field in fig. 42 contains phagocytes and other, presumably sequestered, cells.

In figs. 43 and 44, phagocytosis is marked. Several sinuses have been incorporated into broadened cords, leaving no trace of endothelium and only short segments of basement membrane. In the sinuses that remain, the endothelium persists to varying degrees. At the arrows it is absent. Cell membranes are not sharp, possibly due to oedema.

These tissues were fixed in osmium tetroxide, embedded in methacrylate, sectioned at about 2μ , stained with PAS-H. Figs. 38 and 42, $\times 950$; remaining figures, $\times 1100$.

PLATE 10. Argyric rat spleen

Figs. 45 and 46 are electron micrographs of argyric red pulp, $\times 13,000$ and $\times 65,000$. Note the dense particles of silver in the basement membrane (arrows). In fig. 45 note the order from above down: lumen, endothelium, basement membrane, endothelium, lumen (this one marked by '*L*'s'), endothelium, basement membrane, endothelium. The structure at the lower margin is an endothelial nucleus (*N*) against which, on the right, a mitochondrion lies. Within the broad luminal area in the middle of the photograph are numerous endothelial projections. In addition to its concentration in the basement membrane, silver is also concentrated in intracellular inclusions as in fig. 46. Sections were prepared with Porter-Blum microtome and photographed in RCA-ZE electron microscope.

Figs. 47 and 48 illustrate argyric rat red pulp after phenylhydrazine. In fig. 47 the field represents almost a pure concentration of phagocytes. A sinus, with phagocytic endothelium, persists, recognized by its basement membrane (arrow). Here and there short strands of basement membrane persist. In fig. 48 at least one sinus may be identified (arrow), but the reaction is similar.

The tissue in figs. 47 and 48 was fixed in osmium tetroxide, embedded in methacrylate and stained with PAS-H. $\times 1100$.

Compare this plate with Pl. 1.

OBSERVATIONS ON THE FINE STRUCTURE AND HISTOCHEMISTRY OF THE CAROTID BODY IN THE CAT AND RABBIT

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The fine structural appearances of carotid body glomus cells, first illustrated by Lever and Boyd (1957), have since been further reported on by Garner & Duncan (1958) and by Hoffman & Birrell (1958). In this earlier description (Lever & Boyd, 1957) attention was directed to the presence within the glomus cells of (0.05–0.15 μ) dense membrane-bound granules the population of which varied from cell to cell; and a parallel was drawn with appearances in the adrenal medulla (Lever 1955), the cells of which contain comparable but larger granules. In fact it was postulated that these granules in the carotid body glomus cell might represent the stored form of some humoral agent subserving a local physiological role, possibly as an activator of the Hering's nerve terminals. In the present paper we present evidence which gives further support to this view.

Good evaluations of the earlier writings on the histology and cytology of the carotid body may be found in the works of Hollinshead (1940), De Castro (1951) de Kock (1954) and Adams (1958).

MATERIALS AND METHODS

The rabbit carotid body lies medial to the upper end of the common carotid artery and is closely applied to the adventitia of this vessel; in the cat the relatively larger carotid body is loosely associated with the internal carotid artery immediately above the bifurcation of the common carotid artery.

The general histology and fine structure of the normal carotid body was studied in 6 rabbits and 2 cats, the material being prepared for microscopy as follows. After osmic fixation (see below) tissues were dehydrated through increasing concentrations of ethanol and then methacrylate-embedded. Sections exhibiting silver-gold interference colours were examined by a Siemens electron microscope: thicker (1–2 μ) sections from the same block were examined by phase contrast microscopy in order to identify tissue components. Some of these thicker sections were also attached to slides by albumen coagulation and after removal (in benzene) of the plastic were stained with haematoxylin and eosin.

During most of this investigation carotid bodies were removed under light nembutal or ether anaesthesia supplemented if necessary by local injection of 2 % procaine. Fixation for electron microscopy was by immersion in Dalton's 1 % dichromate osmic solution for two hours. It was later realized that better preservation of certain organelles could be obtained by direct perfusion of fixatives into the common carotid artery of the living animal immediately on the establishment of anaesthesia

by intravenous nembutal and with the minimum of delay or operative interference: and after its subsequent removal, fixation of the carotid body was completed by immersion in Dalton's fluid or other fixative.

Unilateral excision of the superior cervical ganglion and upper sympathetic chain was performed on 6 rabbits and after a week the animals were sacrificed and both carotid bodies removed and prepared for electron microscopy.

The effect of reserpine on carotid body cytology was investigated as follows. Fur clipping and other skin preparation was carried out on two male adult sibling rabbits on the day preceding the experiment. The animals were kept in a quiet place and received $\frac{1}{2}$ mg. largactil/lb. body wt. some 6 hours before operation. One animal then received (intravenously) $\frac{1}{2}$ mg. reserpine in a mixture of ethanol, propylene glycol and water (1:1:2) while the other (control animal) was given an equal volume of this vehicle alone. 30 min. after injection both animals were anaesthetized by a large intravenous dose of nembutal; and rapid carotid perfusion of Dalton's fixative was performed before the carotid bodies were removed.

For the histochemical studies a total of 24 carotid bodies was used, 16 from rabbits, 6 from cats or kittens and 2 from calves. As in the preparation of material for electron microscopy, some rabbit carotid bodies, used in the later stages of the investigation, were fixed by perfusion. Most of this material was fixed as follows: either in 10% neutral formalin or in a modified Orth's fluid containing 2.25% $K_2Cr_2O_7$, 0.25% K_2CrO_4 and 10% AnalaR formalin which was added immediately before use. A few carotid bodies were fixed specially for particular histochemical tests, and these are noted where relevant in the observations. Most specimens were embedded in paraffin wax, but a few were embedded in Nonex 63B (Miles & Linder, 1952) and some in ester wax (Steedman, 1947). All the histochemical tests used to study the carotid body were also performed on similarly prepared sections of intestine and/or adrenal.

1. *Basophilia*. The staining of sections by dilute solutions of methylene blue was followed over the pH range of 1.8–6.0. The procedure used was essentially that discussed by Singer (1952) except that appropriate mixtures of hydrochloric acid, formic acid and sodium acetate (Lewis, 1959) were used to obtain the desired pH values.

2. *Carbohydrates*. The procedure recommended by Lillie (1954) was used for the periodic acid-Schiff test. The original suphation technique of Kramer and Windrum (1954) and the recent modification of Lewis & Grillo (1959) were also used.

3. *Lipids* were visualized by treating sections with solutions of fat-soluble dyes in propylene glycol, according to the procedure of Chiffelle & Putt (1951). Best results were obtained with Sudan Black B and with Fettrot 7B. In addition to the routine formalin-fixed material, use was also made of material fixed in Zenker-formol and post-chromed for two days.

Baker's acid haematin solution was used both on frozen sections as prescribed by him for the detection of phospholipids (Baker, 1946) and on paraffin sections of the post-chromed Zenker-formol material which had been mordanted for an extra hour in dichromate-calcium after taking to water.

4. *Silver reduction* was studied by both the Masson and the Gomori-Burtner silver methods quoted by Lillie (1954) as being suitable for the demonstration of argentaffin

cells. Gold toning and counterstaining were not always carried out and the incubation of some of the carotid body sections was prolonged. The methods developed for the staining of nerve fibres by Bodian (1936) and by Holmes (1947) were also used.

5. *Ferricyanide reduction.* Sections were incubated at room temperature for 15–20 min. in solutions containing 0.5 % ferric chloride and varying amounts of potassium ferricyanide. Molar ratios of ferric to ferricyanide ions between 2:1 and 15:1 were tried as recommended by Lillie and Burtner (1953).

6. *Coupling with diazonium salts.* Sections were incubated at room temperature with a solution of the stable diazotate of 5-chloro-*o*-toluidine (1 mg./ml.) in M/10 tris (hydroxymethyl) aminomethane buffer at a pH of 8.1 for a period of 10–15 min. (intestine) and for up to an hour (carotid body). The diazo-safranin method of Lillie, Burtner & Henson (1953) was also used.

7. *Chromaffin reaction.* Several chromate-containing fixatives were used in attempts to demonstrate this reaction. Their actual composition and mode of use are most appropriately discussed together with the results obtained. Some sections of this chromate-fixed material were mounted unstained, or only lightly stained with haematoxylin. Other sections were stained by the modified Sevki method of Nordenstam & Adams-Ray (1957).

OBSERVATIONS

Histology of rabbit and cat carotid bodies

The rabbit carotid body is lobulated (Pl. 1, figs. 1, 3; Pl. 2, fig. 8), each lobule consisting of parenchymal cells grouped around blood sinusoids and a small amount of fine connective tissue which, for the most part, is perisinusoidal in distribution. Around each lobule is a stroma of connective tissue containing relatively large blood vessels and nerve bundles, but the ultimate distribution of nerve fibres in the lobules is not discernible in haematoxylin and eosin preparations. Many of the small arteries at the periphery of the lobules display 'epithelioid' thickenings of their walls and electron micrographs indicate that in these special areas their endothelium is surrounded by a cuff of somewhat rectangular-shaped elements which are, or resemble, smooth muscle cells. By comparison the cat carotid body exhibits a less noticeable lobulation but is permeated by a similar neuro-vascular stroma which is freely admixed with parenchymal elements: these are grouped around blood sinusoids as in the rabbit.

The parenchymal glomus cells of the rabbit carotid body (Pl. 1, fig. 1) show a light-dark variation in cytoplasmic staining (with haematoxylin and eosin): the darker cells in general were smaller than the lighter ones. The cytoplasm of the latter cells is often 'foamy' or vacuolated in appearance (Pl. 1, fig. 1). While this light-dark cell variation in the parenchymal cells has been observed with regularity in the normal rabbit carotid body it is not such an obvious feature in the cat. The exact relationships of the parenchymal cells to the blood vessels in the carotid body of each species are obscure by light microscopy. In some situations the glomus cells are directly opposed to the walls of the sinusoids (Pl. 2, fig. 6; Pl. 1, fig. 1): in other regions, however, these walls appear thickened by an additional cell with an elongated

nucleus (Pl. 1, fig. 1 b). Whether the cells of this last type are part of the vascular endothelium or constitute adventitial elements lying outside this layer, cannot be determined with certainty by light microscopy.

Electron microscopy of normal rabbit and cat carotid body glomus cells

There is a wide range of appearances of the normal glomus cells in both cat and rabbit but particularly in the latter: perhaps this has its counterpart in the light-dark staining variation observed in these cells by light microscopy (Pl. 6, fig. 15).

Some glomus cells (Pl. 3, fig. 9; Pl. 4, fig. 10; Pl. 6, fig. 15) contain a relatively electron-opaque background cytoplasm in which are compact mitochondria, some of the small ($\pm 150 \text{ \AA}$) particulates probably indicative of ribonucleic acid (Palade 1955) and numbers of larger osmiophile granular bodies to which more attention will be given later.

In contrast are cells (Pl. 6, fig. 15) of much lower general electron opacity which contain vacuolated distended mitochondria and only a small number of these osmiophile granular bodies. There are a number of cell forms intermediate between these two extremes which are generally characterized by some degree of mitochondrial disorganization and a moderate to scant granule content. We also observed a range of cells with a very variable granule content but in which the mitochondria are normal and compact. This category extends from a virtually agranular cell with a low density cytoplasm to one with a high general density and granule content (Pl. 5, fig. 14; Pl. 6, fig. 15).

These various cell forms may be an expression of different phases in a cyclical cell process perhaps of a secretory nature, a suggestion which receives further attention in the Discussion.

The endoplasmic reticulum (Palade & Porter, 1954) of the glomus cell is usually simple in form. It consists of small rough-walled sacs and tubules. However in the cells containing compact mitochondria and few or no osmiophile granules some of the endoplasmic reticulum is arranged in bilaminar sheets (Pl. 4, fig. 11). In the cat glomus cell the outer lamina of the nuclear membrane is often observed to sacculate towards the cytoplasm, the outer wall of these nuclear blebs bearing numbers of cytoplasmic Palade (1955) granules. As in the parathyroid (Lever, 1957, 1958), in which similar appearances have been reported, the nuclear blebs resemble the larger saccular elements of the endoplasmic reticulum. Compact and seemingly discrete paranuclear collections of Golgi membranes are a normal feature of both the light and dark glomus cells.

A constant finding in all carotid bodies examined has been the presence of the osmiophile granular elements previously referred to (Pl. 3, fig. 9; Pl. 4, figs. 10, 12; Pl. 5, fig. 14); these are quite distinct from the much smaller ribonucleic acid particulates of Palade (1955). As already stated, the population of these granules per unit area of section is variable from one glomus cell to another (Pl. 6, fig. 15) but in general is scant in cells of low electron density. Variations in electron density within individual granules and from granule to granule are common (Pl. 4, fig. 12). Very often granules are seen to be surrounded by a continuous thin (100 \AA) line; an appearance suggestive of a membranous investment (Pl. 4, fig. 12; Pl. 3, fig. 9). It has already been suggested (Boyd, Lever & Griffiths, 1959) that these membranes are golgiform.

Granules of comparable electron microscopic appearance have already been described in the rat adrenal medulla (Lever, 1955); but whereas the adreno-medullary granules range in size from 0.05 – $0.4\ \mu$, those in the carotid bodies examined in the present series are between 0.05 and $0.15\ \mu$ in size. The carotid body granules are readily distinguishable from such larger droplets (Pl. 3, fig. 9; Pl. 6, fig. 15), which have a sparse and apparently random distribution in the glomus cell and are very likely of a lipide nature.

The most striking cytological differences between the carotid glomus tissue of the rabbit and cat are: (1) the greater preponderance in the latter of agranular (or seemingly agranular) glomus cells; (2) the darker, more electron dense cell with a heavy content of granules is more commonly observed in the rabbit carotid body; (3) the blebbing of the outer lamina of the nuclear membrane commonly observed in the cat glomus cell has only infrequently been found in the rabbit.

The sinusoids and perisinusoidal spaces

The endothelium of the sinusoids conforms to Palade's (1953) description of the fine structure of capillary endothelium in many other tissues. Imbrication or overlap of contiguous endothelial cells is a frequent appearance as are areas of extreme endothelial thinness in the sinusoid walls, and inward projections of endothelium in the form of 'baffles' are commonly seen (Garner & Duncan, 1958). A well marked space surrounds the sinusoids (Pl. 4, fig. 10; Pl. 5, fig. 14). In general the glomus cells are separated from the endothelium of the sinusoids by this perisinusoidal space containing collagen (Pl. 4, fig. 10) and lined on both sides by a basement membrane applied to the contiguous walls of glomus and endothelial cells. In such perisinusoidal spaces there may be found myelinated and unmyelinated nerves (Pl. 5, fig. 13; Pl. 4, fig. 10), numerous collagen bundles and fibroblasts, in addition to cells of questionable identity which are seen to be in close apposition to the outer endothelial surface (Pl. 5, fig. 14). Although occurring in both cat and rabbit carotid bodies such cells (pericytes) have been more often observed in the latter. The pericytes have an elongated nucleus, a relatively lucid cytoplasm containing few mitochondria and an inconspicuous form of endoplasmic reticulum. They possess none of the osmiophile granules associated with the typical glomus cell. These pericytes differ further from the typical glomus cell in that they are more closely related to the vascular endothelium, being in contact with the endothelial basement membrane (Pl. 5, fig. 14); but sending out cytoplasmic processes between the glomus cells to an unknown varying extent. Collagen fibres are present not only in the perisinusoidal space but are also found to a variable extent between the glomus cells, which may thus be separated by collagen fibres, fine cytoplasmic processes from the pericyte cells and at times by nerve bundles: but very often their plasma membranes are opposed across a narrow intercellular interval containing no defined structure. Dovetailing and interdigitation between cytoplasmic processes of adjacent glomus cells is commonly seen.

Nerve fibres in the carotid body. Bundles of myelinated and unmyelinated axons are frequently seen in perisinusoidal and intercellular spaces (Pl. 3, fig. 9; Pl. 4, fig. 10; Pl. 5, fig. 13). There is no evidence of nerve terminations in relation to vessel walls or pericytes but in a number of instances direct apposition of axonal and glomus cell

plasma membranes have been observed across a narrow (300 Å) interval (Pl. 3, fig. 9). At or near such situations the axoplasm may contain a concentration of mitochondria and numbers of microvesicles, features which are now generally regarded as indicative of nerve terminations.

The rabbit carotid body after sympathectomy. No changes were observed in the parenchymal elements of the carotid body nor in the nerve endings upon the glomus cells following sympathectomy.

The rabbit glomus cells after reserpine administration. Comparison of the fine structure of these cells in the normal control animal with appearances in the identically treated animal which had received reserpine (Pl. 6, figs. 15, 16) strongly suggests that this drug results in a rapid depletion of the specific granular bodies from the glomus cells. It may be significant to recall that Dontas (1957) has demonstrated a marked chemoreceptive stimulation of the carotid body mechanism following reserpine administration.

Histochemical observations

None of the structures in the cat or rabbit carotid body shows marked basophilia. At pH values between 5 and 6 the cytoplasm of many of the glomus cells stains faintly with methylene blue, but this rapidly decreases as the staining pH is reduced below 5. In one carotid body examined, from a cat which was older than any of the other animals studied, there were occasional cells containing granules which stained metachromatically even at the most acid pH used: these were probably mast cells. There are many other reports of mast cells occurring in the perilobular connective tissue of the carotid body (see Adams, 1958). With the periodic acid-Schiff technique many of the connective tissue elements were well stained, but the glomus cells remained unstained. Metachromatic staining after sulphation shows up the glomus cells in high contrast—as completely clear areas in the midst of the densely staining connective tissue. Since this reaction is an extremely sensitive test for polysaccharides such substances must be virtually absent from the cytoplasm of the glomus cells.

It is clear from their electron-microscopic appearance (Pl. 3, fig. 9; Pl. 6, fig. 15) that the glomus cells contain appreciable amounts of lipid. Relatively little of this lipid appears to survive paraffin embedding after the ordinary formalin fixation. However, in the post-chromed, Zenker-formol fixed material, most of the glomus cells do show obvious staining both with Sudan Black B and with Fettrot 7 B (Pl. 2, fig. 5). Treatment with acetone very rapidly removes all this coloration which must therefore be due to physical staining of lipids. Though their sudanophilia was much less than that of cells in many other tissues, such as the intestine and adrenal cortex, it was sufficient to show up quite clearly the cords of glomus cells against the virtually unstained background of the other carotid body tissue components except, of course, the myelin sheaths of nerve fibres which were intensely stained. There was some variation in the sudanophilia between the individual glomus cells in a single section, and under high power this lipid staining often gave a granular or particulate appearance to the cytoplasm. With post-chromed material (both the Zenker-formol paraffin sections and the formol-calcium frozen sections), treatment with the acid haematin solution gave some staining of the glomus cells which was, however, not detectable

after differentiation in borax-ferricyanide for the 18 hours prescribed by Baker (1946) in his method for the identification of phospholipids. After such differentiation, in fact, myelin was the only lipid component which remained stained.

Phenolic amines in the glomus cell cytoplasm. The osmiophile granules in the cytoplasm of the glomus cells have an electron-microscopic appearance reminiscent of two other types of cytoplasmic granules whose chemical identity is fairly firmly established: (a) those of the adrenal medulla (Lever, 1955) known to contain catechol amines or their precursors (Blaschko, Hagen & Welsh, 1955) and (b) those of the argentaffin cells of the intestine (Christie, 1955) thought to contain 5-hydroxytryptamine or some closely related substance (Erspamer, 1954). An attempt was therefore made by histochemical methods to discover if any of these or similar substances were present in the glomus cells of the carotid body.

Phenolic amines, such as adrenaline, noradrenaline and 5-hydroxytryptamine, are reducing substances and should give a positive reaction with a number of histochemical tests. Thus they should reduce ferricyanide to give a positive Schmorl reaction and silver ions to give a deposit of metallic silver. Adrenal medullary cells and certain cells in the intestinal mucosa give both these reactions under the appropriate conditions and can thereby be rendered clearly visible in tissue sections. In material fixed by immersion in formalin the glomus cells gave only a faintly positive Schmorl reaction, but after perfusion and fixation with chromate the cords of glomus cells showed up quite clearly amongst the much more faintly stained connective tissue (Pl. 2, fig. 7). With the Masson and the Gomori-Burtner silver methods only faint staining of the glomus cells was obtained in a period sufficient to produce marked blackening of cells in the adrenal and in the intestine. However, with the Bodian technique, the glomus cells were often intensely stained (Pl. 2, fig. 4).

Another histochemical test with a high specificity for phenolic compounds is the reaction with diazonium salts at an alkaline pH to give brightly coloured azo dyes. With the diazonium salt of 5-chloro-*o*-toluidine, argentaffin cells in the intestine rapidly acquire a fiery orange colour which is rather less intense after chromate fixation than after neutral formalin: under the same conditions adrenal medullary cells stain a deep brownish-orange after chromate fixation but only a much fainter yellowish-orange after neutral formalin, and glomus cells of the carotid body stain a definite orange-yellow after chromate but only a very faint yellow after formalin. Although the staining of the glomus cells was quite distinct (Pl. 1, figs. 2, 3) it was considerably less intense than that of adrenal medullary cells fixed under the same conditions and was more yellow in colour.

A very sensitive, though somewhat unspecific, test for phenolic amines is the production of yellow or brown pigment by chromate-containing fixatives—the classic chromaffin reaction. With this test the glomus cells give a definite yellow or brownish-yellow coloration which is, however, much fainter than in adrenal medullary cells and lacks visible granularity. Several fixation procedures were tried, both with carotid body and with adrenal. The buffered chromate-dichromate mixture recommended by Hillarp & Hokfelt (1955) gave good pigment formation but poor preservation of cytological detail. The fixative finally chosen, that given in *Material and Methods*, was a modified buffered Orth's fluid and its use resulted both in good pigment formation and cytological preservation. In carotid bodies perfused with

this fixative, staining of the glomus cell cytoplasm is marked and is particularly obvious when sections are viewed or photographed with blue light (Pl. 2, fig. 8). With the modified Sevki method of Nordenstam & Adams-Ray (1957) Giemsa gives good differential staining of the glomus cells (Pl. 2, fig. 6). The mechanism of this staining is not known but it appears to be related to the presence of catechol amines since the staining is much less in adrenals from reserpine-treated animals (Lever & Lewis, 1959).

DISCUSSION

The results of the various histochemical tests strongly suggest the presence within the glomus cell cytoplasm of a phenolic amine or some closely related chemical substance. Admittedly, some of the most positive indications were obtained with tests which probably have only a poor specificity for phenolic amines, but this is to be expected from the known sensitivities of the various tests used. In fact, the relative order of staining intensities obtained with these tests was strikingly similar in the glomus cells of the carotid body and in the medullary cells of the adrenal. The absolute intensity of staining, however, was much less in the carotid body than in the adrenal; it is obviously dangerous to make a quantitative estimate from purely visual comparisons, but the staining of the glomus cells appeared to be less by a factor of at least ten. Because of this faint staining of the glomus cells it is therefore not possible to identify the phenolic amine present with certainty by existing histochemical methods. The available evidence, however, appears to be more in favour of its being adrenaline or noradrenaline rather than 5-hydroxytryptamine.

However likely may be the relationship of this histochemical staining in the glomus cells to the presence of granular bodies (as observed in electron micrographs) it cannot be directly proved, since owing to their very small size—none being larger than $0.15\ \mu$ —these bodies are not discretely observed in the light microscope, a fact which would account for the general coloration and absence of 'granularity' in both the chromaffin and diazonium staining. It is also not certain whether these osmiophile granules are identical with the fuchsinophilic and siderophilic granules observed by Hollinshead (1943), and Ross (1957). It is possible that ordinary histological processing may cause the small osmiophile granules to swell or to aggregate sufficiently to become observable with the light microscope.

The cytological changes observed in the glomus cells after reserpine treatment might also be regarded as evidence of a 'chromaffin' nature for their inclusion granules. As in adreno-medullary cells (Lever & Lewis, unpublished observations) so also in the carotid body glomus cells, this drug produces a striking disappearance of inclusion granules. This is not to imply that these cell types are necessarily analogous but merely to stress that both give a chromaffin reaction and show a similar response to reserpine. Indeed it is not clear that the carotid body glomus cells should properly be called a *chromaffin tissue*, in the usually accepted sense of the phrase, since following sympathectomy no changes were observed in their cytology or in the nerve terminals upon their surfaces. From this one can infer that the glomus cells do not have a sympathetic innervation and should thus not be classed with the adrenal medulla and paraganglia as typical chromaffin tissues.

As a result of these observations we are unable to agree with Garner & Duncan (1958) that the glomus cell granules are an index of faulty fixation. On the contrary we consider they are a normal cell inclusion and one which is very likely concerned in the specific function of the glomus tissue. We hold this view for the following reasons: (i) These granules have been consistently observed under very favourable conditions of preparation. In fact there are always many more of them present in the glomus cells of carotid bodies which are vitally perfused with the osmic fixative before removal than in those (carotid bodies) which are removed after a lengthy operative procedure and secondarily fixed. (ii) In the Golgi region and elsewhere in the cytoplasm the glomus cell granules are frequently found to be individually enclosed by a delicate smooth-walled investing membrane and thus present an appearance similar to that of the secretory granules in the adrenal medulla (Lever, 1955), the endocrine pancreas (Lacy, 1957) and the adenohypophysis (Farquhar & Wellings, 1957). (iii) There is a variation in the cytoplasmic concentration of these granules from one glomus cell to another, a fact which might be interpreted as an indication of different functional levels between these cells. Such heterogeneity is common within secretory tissues and has been described in the parathyroid (Lever, 1958) and the adrenal medulla (Lever, 1959, *in press*). (iv) Following reserpine injection the glomus cell granules are greatly reduced in number as compared with the untreated normal control.

It is not clear what is the nature of the pericytes which are situated in the perisinusoidal spaces and which send cytoplasmic processes to an undetermined extent between the glomus cells. They probably correspond to the type II parenchymal cells described by de Kock (1954) and in their cellular-vascular relationship at any rate they resemble the 'stellate' cells observed by Rhinehart & Farquhar (1955) in the adenohypophysis. These pericytes have a cytoplasm of very 'watery' appearance (Pl. 5, fig. 14) and do not resemble glomus cells, muscle cells, fibroblasts, or neurones. Electron-micrographic comparison between a typical Schwann cell (Pl. 5, fig. 13) and a pericyte (Pl. 5, fig. 14), both within a perisinusoidal space, does not suggest an identity between them, but this must remain an open question. From their position the pericytes might conceivably perform a conductive role from sinusoid to glomus cell.

Without a clearer conception of the significance of these pericytes it would certainly be unwise to make any comprehensive speculations on the cellular mechanism underlying carotid body activity, but we believe that the results of our investigations do point to a humoral or local endocrine role for the carotid body glomus cell, a concept formerly presented by Moulon (1904). Conceivably the membrane-bound cytoplasmic granules in the glomus cell represent 'packets' of a transmitter substance—a phenolic amine, which might be responsible for initiating nerve impulses in the Hering's nerve terminals which are closely applied to the glomus cell walls and have not been found in relation to blood vessel walls or to the pericytes. We consider that the contents of these membrane-bound granules are released from the glomus cell in response to reserpine administration, during operative handling of the carotid arteries, and also in anoxia and hypercapnia, both of which states are certainly encountered during the initial stages of inhalation anaesthesia. Until we appreciated these last points our histochemical and electron microscopic findings were extremely

variable. Consistent results were obtained in the normal living rabbit carotid body only if this organ was perfused with the fixative after cannulation of the common carotid artery with the minimum delay and operative disturbance immediately on effecting anaesthesia by intravenous nembutal. Unless this procedure was followed the results of these staining techniques were inconsistent, a fact which may well account for the differences in opinion on the occurrence or otherwise of a true chromaffin reaction in the carotid body.

Something should perhaps be said about the possible role of acetylcholine in the initiation of chemoreceptor impulses—a topic which has been very adequately reviewed by Heymans & Neil (1958). Intracarotid injection of acetylcholine will initiate chemoreceptor impulses in Hering's nerve and anticholinesterases will potentiate this effect. A number of ganglioplegic drugs will abolish this response to acetylcholine (and that to such alkaloids as lobeline) without, however, blocking the normal chemoreceptive response to anoxia: so it seems unlikely that acetylcholine can be directly concerned in the transmission of the normal chemoreceptor response. It is quite possible that the glomus cells and/or the afferent terminals of Hering's nerve are, incidentally, responsive to acetylcholine, as are many other sensory structures, and that the cholinesterases known to be present in the carotid body (Hollinshead & Sawyer, 1945; Koelle, 1950) are present to guard against the dangers of this responsiveness. Such pharmacological studies do not exclude the possible role of an adrenaline-like substance in the initiation of the normal chemoreceptor response; and the observation by Dostas (1957) that intracarotid injection of small doses of reserpine cause a prolonged burst of chemoreceptive impulses in Hering's nerve would appear to strengthen this view.

Finally, a recent observation of Comline & Silver (1958) is perhaps relevant. They found that the *denervated* adrenal of the foetal sheep secretes noradrenaline in response to anoxia. This direct anoxic response of at least some medullary cells is lost late in foetal life. If the glomus cells of the carotid body retained this property, however, and if the liberated noradrenaline stimulated the neighbouring sensory nerve endings, an explanation would be provided for much of the known physiology of the chemoreceptor mechanism.

SUMMARY

1. It was possible to demonstrate positive staining of glomus cell cytoplasm by the chromaffin, Giemsa's ferric-ferricyanide and diazonium reactions, especially after fixation by intravital perfusion. These histochemical results suggest the presence of phenolic amines in carotid body glomus cells.

2. Parallel electron-microscopic investigation has demonstrated the presence of 0.05–0.15 μ membrane-bound granular bodies in the glomus cell cytoplasm of both rabbit and cat carotid bodies.

3. The population density of these granular bodies varies between glomus cells, and this variation and that of certain other cell features may be indicative of differing states of secretory activity within the glomus.

4. Reserpine administration results in a general depletion of these granular bodies throughout the glomus, a finding in parallel with the reserpine depletion of the 'adrenaline' bodies from the adrenal medulla.

5. Nerve endings have been seen in relation to glomus cells but terminals have not been found on sinusoid walls or in relation to the pericytes which in places separate the vascular endothelium from glomus cells and send processes to an unknown extent between glomus cells.

6. Sympathectomy produces no changes in the glomus cells or the nerve terminals associated with them.

7. These findings are discussed and a local humoral role is tentatively suggested for the glomus cells: possibly the electron-dense membrane-bound granular bodies contained by them represent (as in the adrenal medulla) a stored form of some catechol amine which on release in response to various stimuli (reserpine, anoxia, etc.) may stimulate adjacent Hering's nerve terminals.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Rabbit carotid body (c.b.) lobule. A light-dark cytoplasmic staining variation is observed between glomus cells: some light cells show a 'foamy' cytoplasm (*a*). Occasional spindle shaped cells (*b*) are related to sinusoid walls. Osmic-fixed, methacrylate-embedded specimen stained with haematoxylin and eosin after removal of plastic. $\times 1500$.
- Figs. 2, 3. Rabbit carotid body, chromate-fixed 10μ section treated with diazonium salt, no nuclear stain, photographed with blue light. Note cytoplasmic staining of glomus cells. Fig. 2 $\times 280$; Fig. 3 $\times 1040$.

PLATE 2

- Fig. 4. Rabbit carotid body, formalin fixed, stained by modified Bodian silver proteinate technique to show the cytoplasmic argyrophilia of the glomus cells, nerve fibres and connective tissue unstained. $\times 1600$.
- Fig. 5. Cat carotid body, Zenker-formol fixed and postchromed material, $3\ \mu$ section stained with Sudan Black B in propylene glycol to show the sudanophilia of the glomus cell cytoplasm. $\times 1040$
- Fig. 6. Rabbit carotid body, formol-chromate fixed, $10\ \mu$ section stained by the modified Sevki technique. Erythrocytes (*R*) are stained pink and glomus cells (*G*) purple. $\times 1040$.
- Fig. 7. Rabbit carotid body, formol-dichromate fixed, stained by the ferric-ferricyanide technique. Note staining of the two groups of glomus cells (*G*). $\times 1040$.
- Fig. 8. Rabbit carotid body, formol-chromate fixed, nuclei lightly stained with haematoxylin, photographed with blue light. Note the faint but definite cytoplasmic 'chromaffin' staining of glomus cells (*G*). $\times 780$.

PLATE 3

- Fig. 9. Rabbit carotid body—electron micrograph of a group of glomus cells surrounded by collagen fibres (*c*) and fibroblasts (*F*). Note: Schwann cell (*W*) containing an unmyelinated axon (*a*), nerve endings on glomus cells (*n*), lipid bodies (*L*) and small membrane-bound granular bodies (*g*) in the glomus cell cytoplasm. $\times 15000$.

PLATE 4

- Fig. 10. Note general tissue arrangements in this electron micrograph of rabbit carotid body: sinusoid, *S*; glomus cells, *G*; collagen, *C*; nerve fibres, *n*; endothelial cells, *e*; pericyte, *P*; Schwann cells, *W*. $\times 3000$.
- Figs. 11, 12. Electron micrographs of glomus cells. Note membrane-bound cytoplasmic granular bodies (*g*) and vacuolated mitochondria (*M*) in fig. 12. Figure 11 contains numerous cytoplasmic sacs (*s*) but no obvious granules similar to *g* in fig. 12. Granular endoplasmic reticulum (*ER*) is particularly obvious in fig. 11. Both at $\times 40000$.

PLATE 5

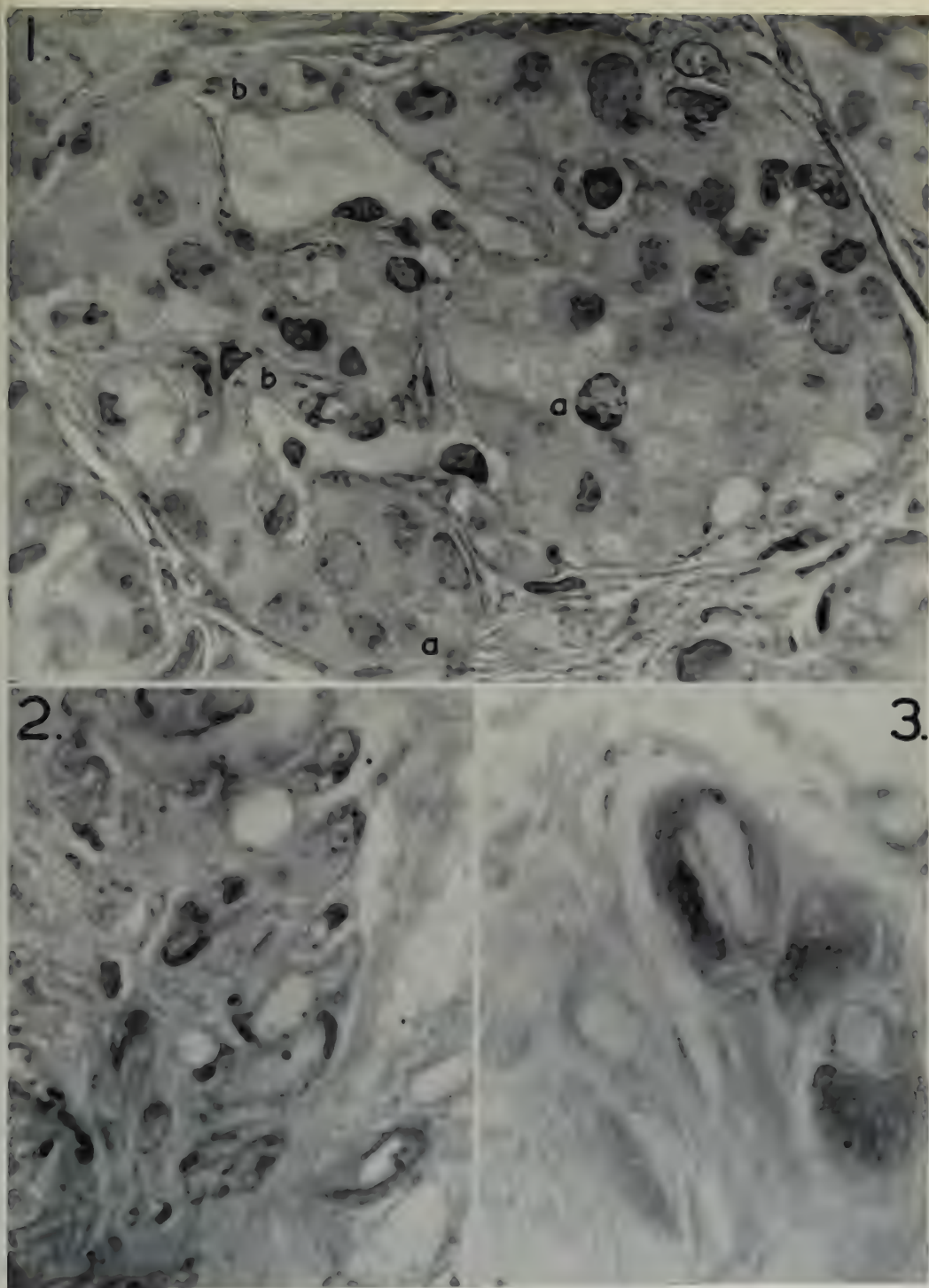
Both figures are electron micrographs depicting typical contents of the perivascular space in the rabbit carotid body.

- Fig. 13. Note unmyelinated axon: *A* suspended by mesaxon (*m*) from surface of Schwann cell (*W*); collagen, *C*; sinusoid endothelium, *E*. $\times 27500$
- Fig. 14. Note glomus cell (*G*) with contained membrane-bound granular bodies; collagen, *C*; a pericyte of questionable nature, *P*; sinusoid endothelium, *E*. $\times 15000$.

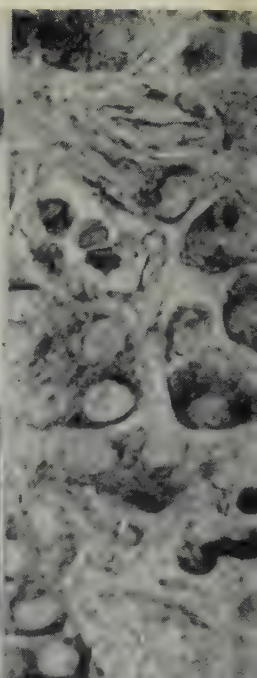
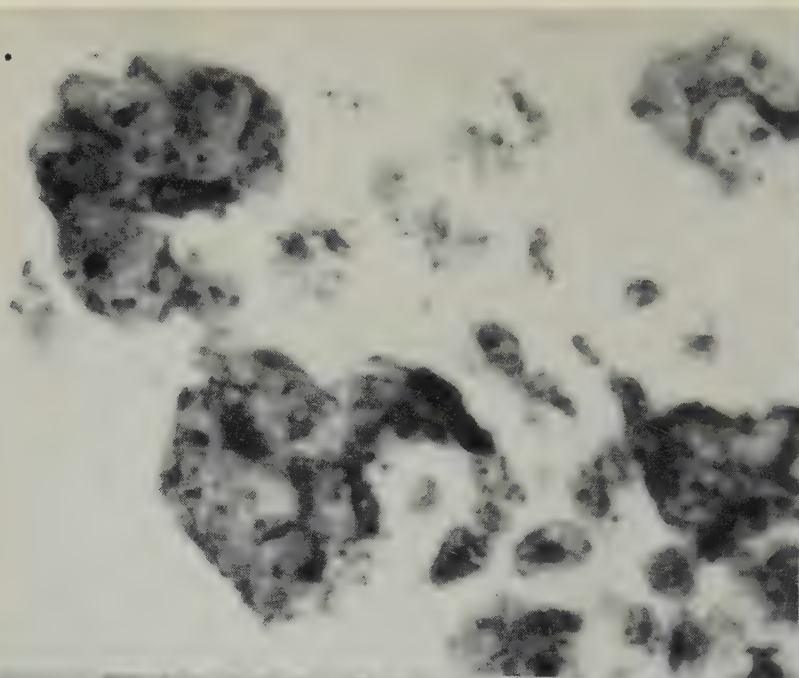
PLATE 6

Two sibling rabbits were prepared by basal narcosis for operation on the following day. One was given reserpine 0.5 mg. (in suitable vehicle) intravenously (fig. 16) while to the other (fig. 15) animal the vehicle only was administered (by i.v.i.).

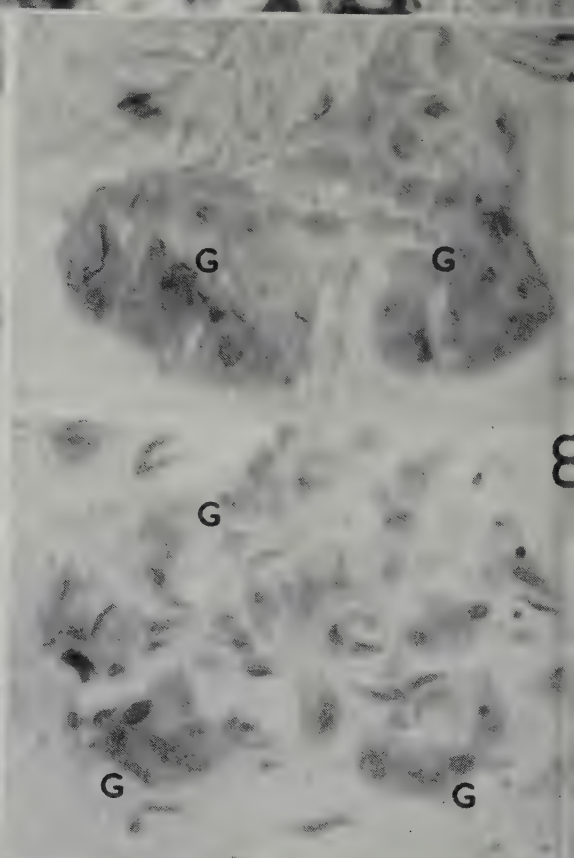
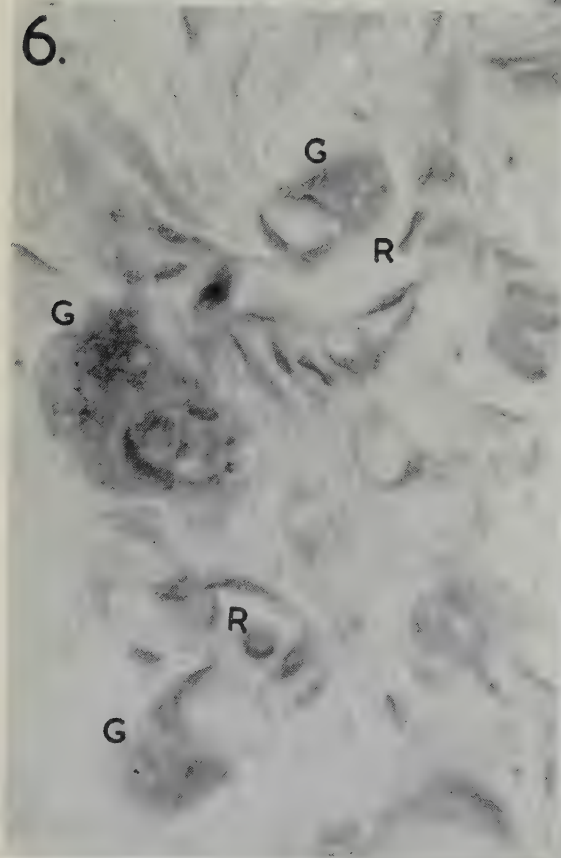
- Fig. 15. Shows the normal cytological variation of the glomus cells. Some, more electron dense, cells (*G*₁) contain numbers of osmiophile granular bodies (*g*) as already depicted in figs. 9, 12, 14 while other glomus cells (*G*₂) contain fewer granular bodies; in further glomus cells (*G*₃) also containing few granular bodies many of the mitochondria are vacuolated. $\times 14000$.
- Fig. 16. After reserpine the normal cytological variations as seen in fig. 15 are not so apparent and most of the glomus cells contain few or no granular bodies. $\times 14000$.

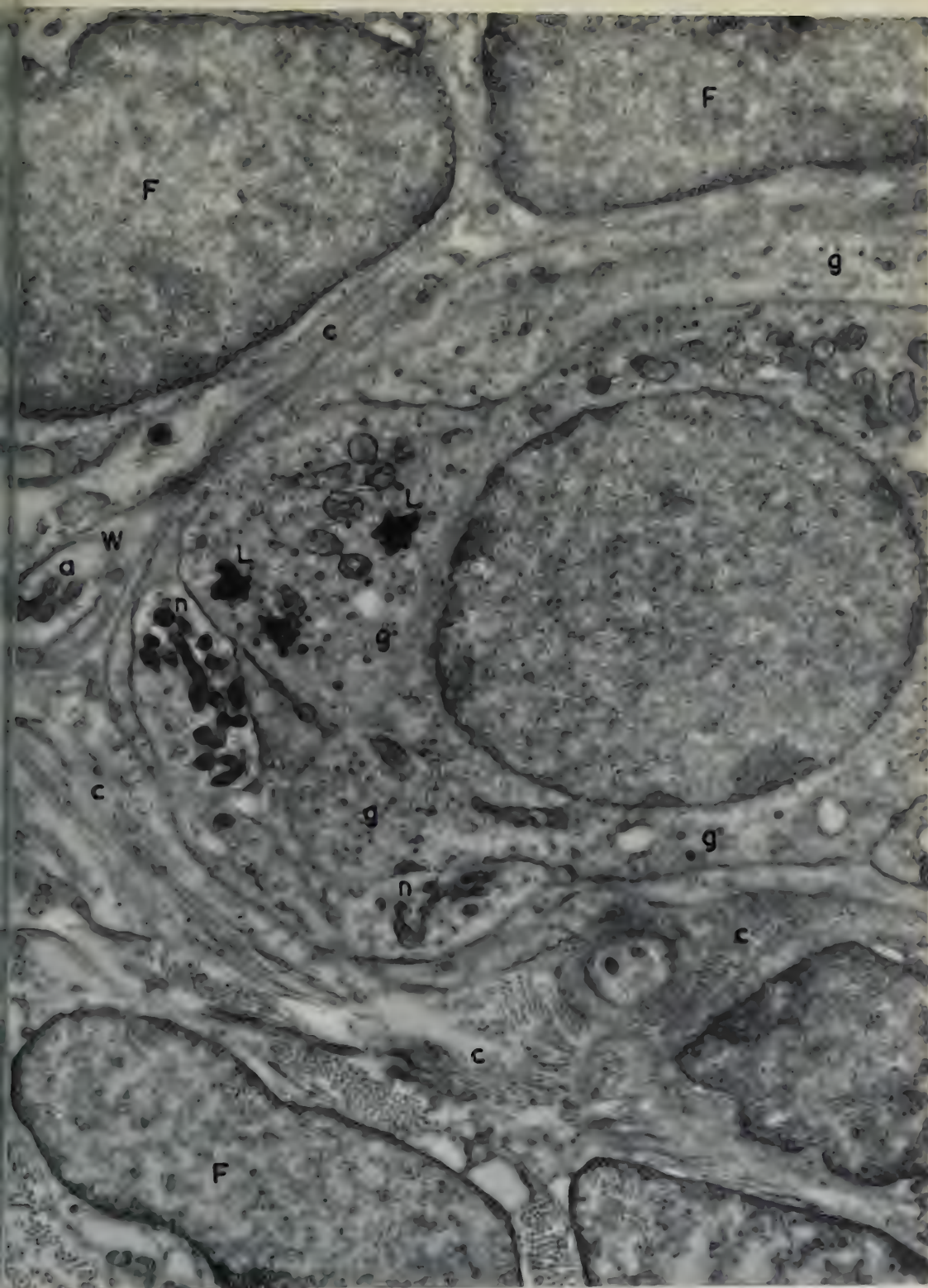


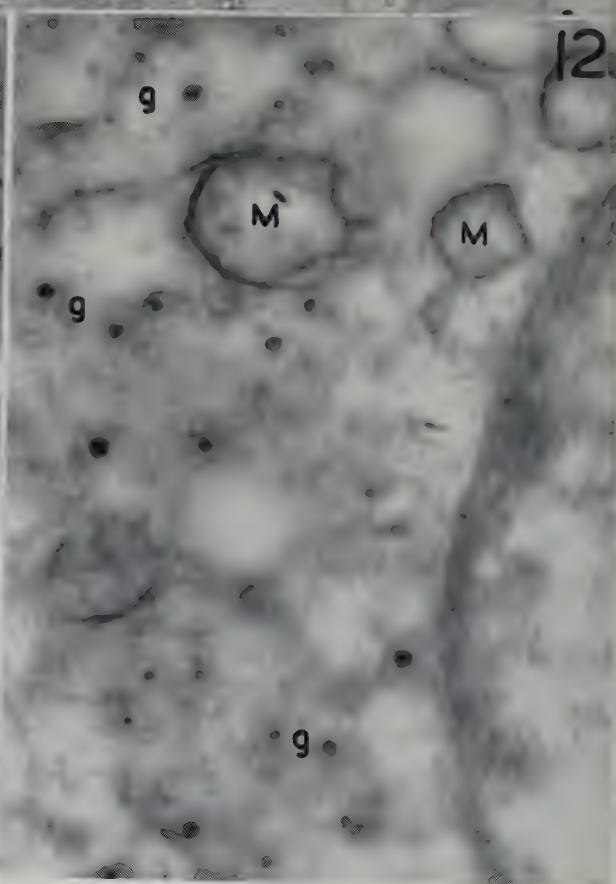
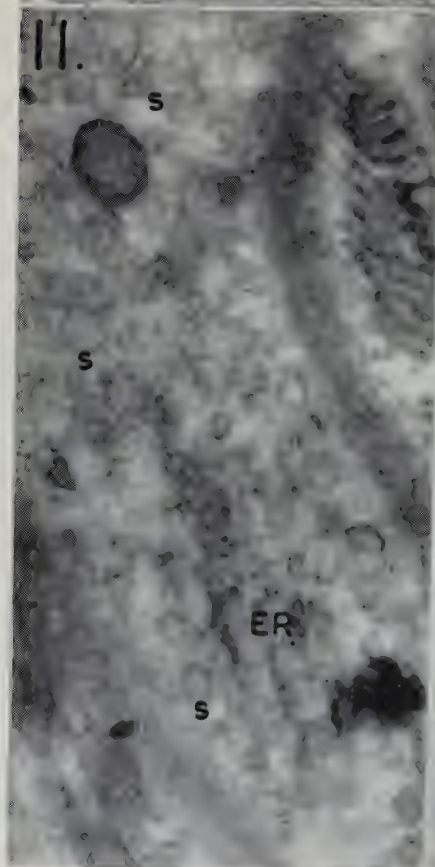
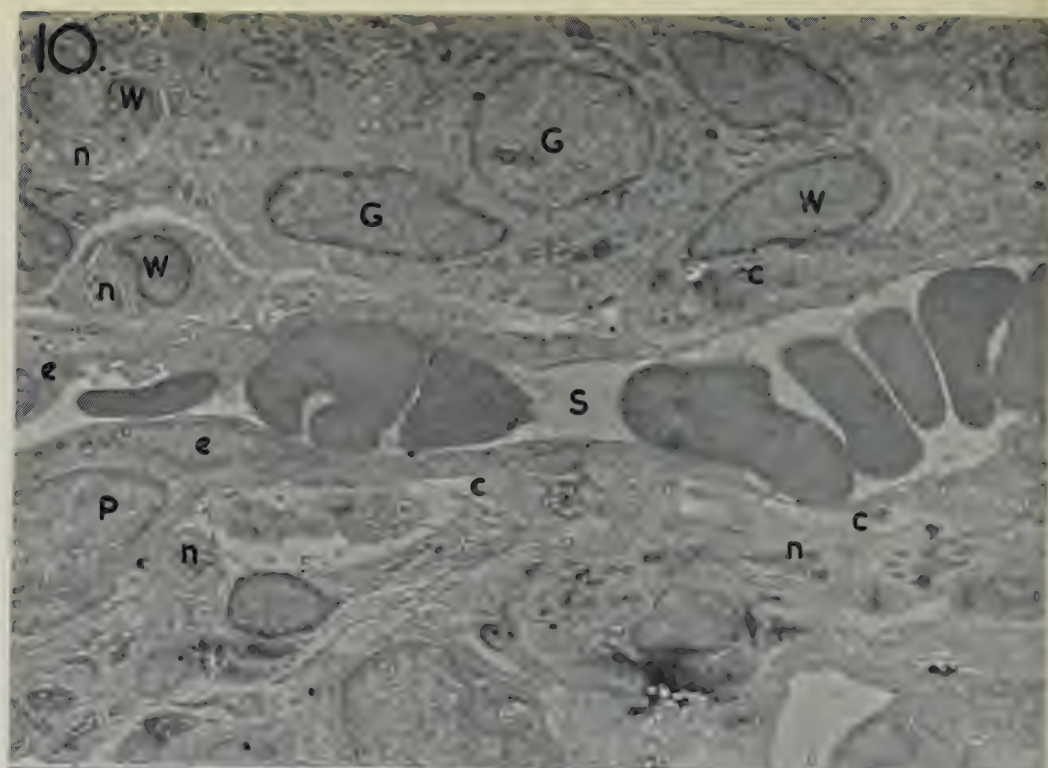
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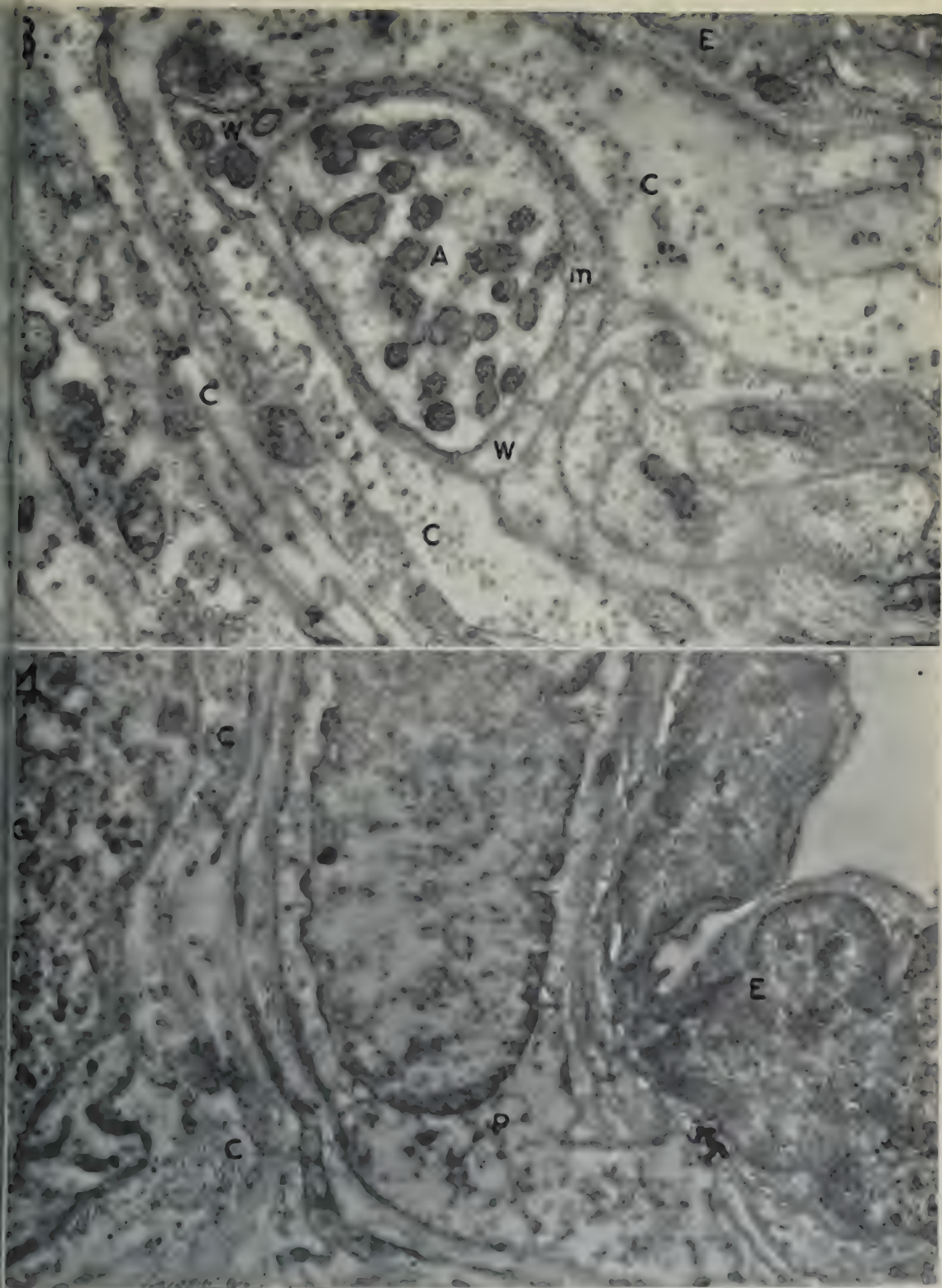


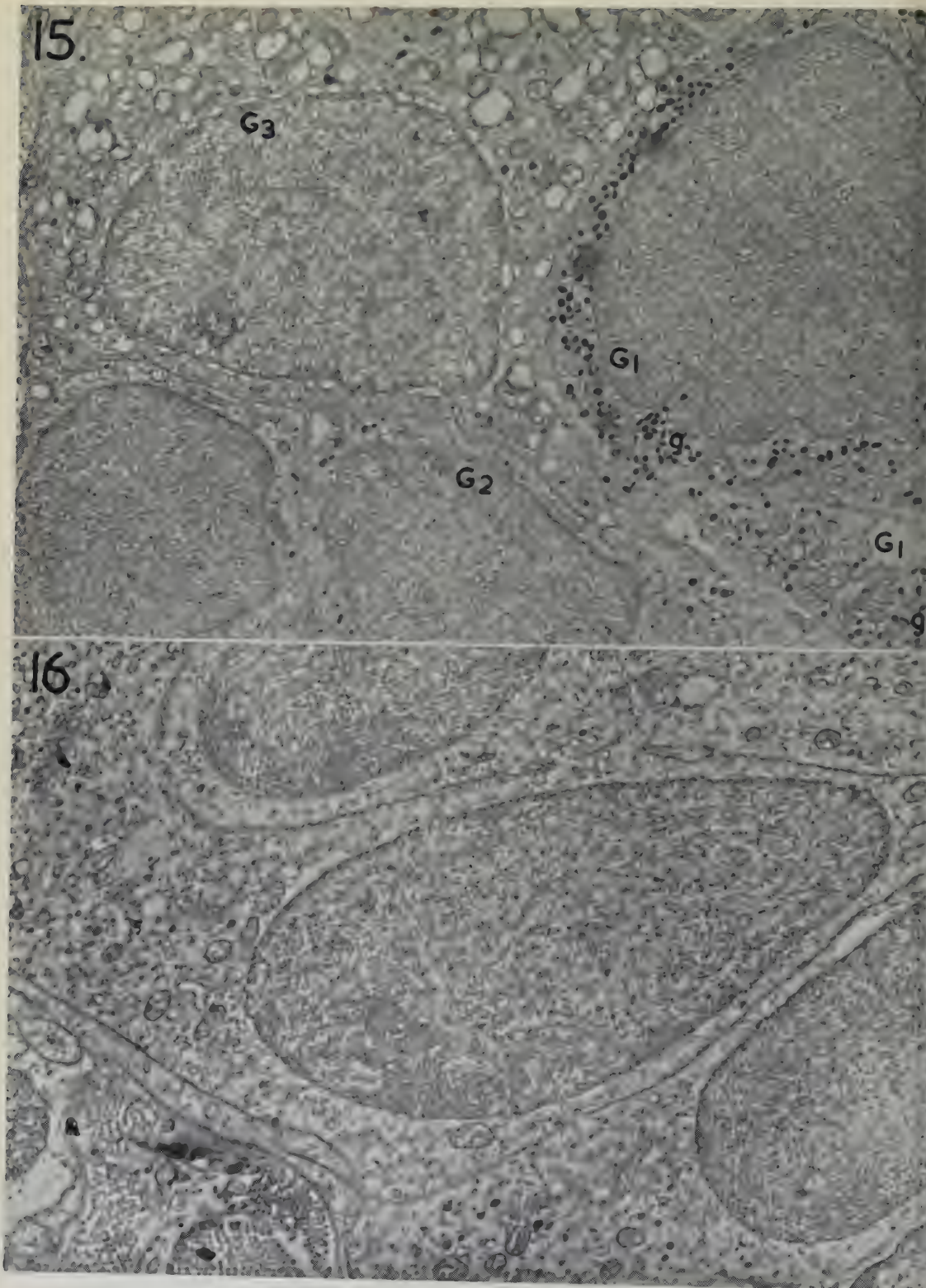
6.











THE EPITHELIUM OF THE UROGENITAL SINUS IN FEMALE HUMAN FOETUSES

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The epithelium of the urogenital sinus is classically described as an endodermal derivative, arising from the ventral division of the cloaca (Felix, 1912). This view was, however, questioned by Zuckerman (1940), who suggested that an ectodermal element was incorporated in the sinus epithelium. He believed that the production of a stratified squamous cell proliferation in response to oestrogenic stimulation was a property of all the epithelium derived from the urogenital sinus. Because a similar response occurred in the epidermis of the sexual skin area, he suggested that the oestrogen-sensitive sinus epithelium must also be partly derived from ectoderm. There was, he pointed out, embryological evidence in certain mammals (Siddiqui, 1937; Barnstein & Mossman, 1938) that the epithelium of the caudal part of the sinus was of ectodermal origin. He considered that the characters of this ectodermal epithelium were impressed upon the more cranially situated endodermal epithelium of the sinus, thus explaining the similarity of the oestrogen responses of the epidermis and the sinus epithelium. Support was lent to Zuckerman's hypothesis by the work of Burns (1942), who found that in pouch young of the opossum an oestrogen-sensitive epithelium gradually grew cranially through the urogenital sinus from the ectodermal cloaca, displacing the original insensitive endodermal lining before it.

If Zuckerman's hypothesis is applicable to the sinus epithelium of the human female, one might expect some indication of affinity, during development, between the sinus epithelium and the epidermis of the area corresponding to the sexual skin. Despite the abundance of literature on the development of the human vagina there is little detailed information on the changes occurring in the sinus epithelium of the female. It was, therefore, decided to make a study of this subject.

The material consisted of the urogenital systems of thirteen female embryos and foetuses, from 28 to 375 mm. C.R. length, and of a 1-month female infant. The younger specimens were already serially sectioned at 6–10 μ and stained with haematoxylin and eosin or a trichrome stain. Some sections of the older ones were also submitted to certain histochemical techniques.

OBSERVATIONS

In the 28 mm. embryo the greater part of the pars pelvina of the sinus is lined by a fairly thick epithelium, consisting usually of six or seven layers of cells with relatively large nuclei and fairly deeply staining cytoplasm (Pl. 1, fig. 1). In the region of the Müllerian tubercle, however, the epithelium is 'differentiated' into basal deeply staining and superficial pale-staining zones, and the subsequent formation of the epithelium of the entire vagina from this small area has been described

elsewhere (Bulmer, 1957). The thick, relatively darkly staining epithelium of the pars pelvina extends down over most of the dorsal wall of the pars phallica, but as it is followed caudally along the ventral wall it gradually changes its character, becoming lower and less darkly staining. This epithelium, sometimes consisting of only three layers of cells, is invaginated along the under surface of the phallus as the urethral plate. At the distal tip of the phallus (Pl. 1, fig. 2) and over the inner aspects of the urethral folds it merges with the perineal epidermis, from which it cannot be clearly distinguished. While, therefore, the epithelium of the pars pelvina is quite different in appearance from the developing epidermis of the perineum there is an intermediate zone between the two, occupying most of the pars phallica, in which the epithelium gradually changes its character.

In 42 and 50 mm. foetuses the darkly staining stratified epithelium still lines the whole of the pars pelvina except for the small area in the region of the Müllerian tubercle. As it is followed into the upper part of the pars phallica it becomes rather thinner and not quite so darkly staining, and is invaginated along the under surface of the phallus as the urethral plate. The inner aspects of the urethral folds are lined by a stratified epithelium of very much more pale-staining cells, with relatively smaller nuclei. This epithelium appears to be identical with the developing perineal epidermis. It forms the distal tip of the glandar urethral plate, and extends cranially through a few sections as a thin layer of cells lying superficial to the more proximal part of the urethral plate, which itself is formed of the more darkly staining cells. The distinction between the pale-staining epithelium at the distal extremity of the pars phallica, which is indistinguishable from the epidermis, and the more darkly staining epithelium which forms most of the urethral plate is now fairly sharp.

At the 65 mm. stage the distinctions between the different epithelia are well established. The small area of 'differentiated' epithelium in the region of the Müllerian tubercle is now proliferating to form the primordium of the epithelial vagina. The rest of the pars pelvina is lined by a darkly staining stratified epithelium, which gradually becomes thinner as it is followed down into the pars phallica. The proximal portion of the urethral plate has become opened out into a shallow groove, which is lined by the thin, darkly staining epithelium. Further caudally (Pl. 1, fig. 3), the invagination which represents the deeper layers of the urethral plate starts immediately behind the corona glandis. It is formed of the same darkly staining epithelium, and on either side of it the ventral wall of the sinus forms a shallow 'secondary' urethral groove (Glenister, 1954), which has arisen by the opening out of the more superficial layers of the urethral plate. The groove is lined by the darkly staining epithelium, and this is very much thinner than the pale-staining stratified epithelium over the inner aspects of the urethral folds, which it meets at a very distinct junction. Further caudally still, the pale-staining epithelium extends across and overlies the darkly staining cells of the urethral plate for a short distance. Eventually, the extreme distal tip of the urethral plate is formed entirely of the pale-staining epithelium.

The appearances and distribution of the different epithelia now remain essentially unchanged until after the 140 mm. stage. The 'differentiated' type of sinus epithelium continues to proliferate as the vaginal primordium. The darkly staining 'undifferentiated' type of epithelium occupies the rest of the pars pelvina, and most

of the pars phallica and urethral plate. The inner aspects of the urethral folds and the distal extremity of the urethral plate are formed of the pale-staining 'epidermal' type of epithelium. For convenience, these terms will be used to designate the various epithelia.

Between the 65 and 140 mm. stages most of the remainder of the urethral plate becomes opened out into a shallow groove, and by the 112 mm. stage the plate itself is entirely confined to the glans. At the 140 mm. stage a tiny remnant can still be identified. The 'undifferentiated' sinus epithelium remains relatively thin, while the 'epidermal' epithelium becomes very much thicker, and the two are always sharply distinguished from each other at their junction (Pl. 1, fig. 4). The lower end of the vagina becomes enormously dilated, and invaginates the upper end of the sinus to form the hymen. The upper aspect of the hymen is covered by the thick, large-celled, stratified vaginal epithelium, while its lower aspect is lined by the thin, darkly staining 'undifferentiated' epithelium, formed of very much smaller cells (Bulmer, 1957). A few sections of the 140 mm. foetus were stained by the periodic acid-Schiff technique, though unfortunately no digestion controls were available. The vaginal epithelium and the 'epidermal' epithelium are rich in PAS-positive material, while the 'undifferentiated' epithelium gives either a very faint reaction or no reaction at all.

At the 180 mm. stage there are several striking changes. The 'epidermal' epithelium has the same distribution as before, but is now thickly keratinized (Pl. 1, fig. 5). Towards the distal end of the glans it is continuous with the glandar lamella. The large part of the vestibule is lined by an epithelium which, except that it is rather thinner, is almost identical with the vaginal epithelium, with no cytoplasmic staining in the intermediate and superficial layers of cells (Pl. 1, fig. 5). While the 'epidermal' epithelium now has a weak PAS reaction, due to small quantities of glycogen, the 'vaginal' type of vestibular epithelium has large quantities of glycogen, confirmed by diastase digestion controls, and 'intercellular walls' like those of the vaginal epithelium itself (Bulmer, 1959), sudanophilic and with a diastase-fast PAS reaction (Pl. 1, fig. 6). On the under surface of the proximal part of the glans the distal extremity of the 'vaginal' type of epithelium is associated with a tiny invagination which seems to be a remnant of the urethral plate (Pl. 2, fig. 7).

There is, however, a further type of epithelium in the vestibule at this stage. This is usually a low, darkly staining stratified epithelium, rather similar to the 'undifferentiated' epithelium of the earlier stages (Pl. 2, fig. 8). Occasionally the cells are arranged in a stratified columnar pattern, and there are also small alveolar gland spaces, lined by columnar cells with a diastase-fast PAS reaction, both in the epithelium and just beneath it. This 'glandular' type of epithelium, of which the gland spaces are presumably precursors of the lesser vestibular glands, occupies a strip in front of the urethral orifice and several small areas in the posterior part of the vestibule, where it is intermingled with areas of the 'vaginal' type of epithelium.

At the 375 mm. stage the 'epidermal' epithelium is very thickly keratinized (Pl. 2, fig. 9), and is continuous with a similar hypertrophic epidermis over the perineum. The keratin of this 'sexual skin' area is very much thicker than that of the epidermis over the rest of the body. The 'epidermal' epithelium contains glycogen only in very small quantities in a few scattered areas, and apart from this the PAS reaction is

completely negative. The 'vaginal' type of vestibular epithelium is rich in glycogen, and 'intercellular walls' are well marked. The 'glandular' epithelium is commonly of stratified columnar form, alveolar spaces are very frequent and their lining cells give a diastase-fast PAS reaction (Pl. 2, fig. 10).

In the vestibule of the 1-month-old infant the epithelial types are much less distinct. Like the vaginal epithelium, the 'vaginal' type of vestibular epithelium is much more darkly staining than in the foetal material, with cytoplasmic acidophilia of the intermediate and superficial layers of cells (Pl. 2, fig. 11). 'Intercellular walls' can be demonstrated only very faintly and there is little glycogen, though rather more than in the vaginal epithelium itself. The 'glandular' epithelium is almost non-existent, and in an extensive series of sections through the posterior part of the vestibule only one glandular alveolus was found. The lining cells of this, however, still give a diastase-fast PAS reaction. The 'epidermal' epithelium, like the surrounding sexual skin, is very much thinner than before, is often without a superficial layer of keratin, and shows more extensive glycogen deposits than in the 375 mm. foetus.

DISCUSSION

The material which has been studied does not include sufficiently early stages for a direct study of the origin of the sinus epithelium. However, the conditions in the 28 mm. embryo, which do not appear to correspond exactly with Glenister's description (1954), suggest that a histological investigation of early material may not answer this problem. In the 28 mm. embryo it is impossible to make any sharp distinction between the pale-staining epithelium of the pars phallica, which includes the whole urethral plate, and the developing epidermis of the perineum. Nor is there a sharp junction between the pale-staining epithelium of the pars phallica and the darkly staining epithelium of the pars pelvina. The epithelia are not well differentiated from each other at the 28 mm. stage, and it is not until the 65 mm. stage that the clear distinction can be made between the pale-staining 'epidermal' epithelium and the darkly staining 'undifferentiated' sinus epithelium. The distribution of the latter is much more extensive than that of the darkly staining epithelium in the 28 mm. embryo. Histological examination does not establish the relations between the epithelia which are present at the 65 mm. stage and those which can be identified in the 28 mm. embryo. There would probably be little value, therefore, in pursuing this into still earlier stages.

Once they are established, however, the differing developmental characteristics of the 'epidermal' epithelium and the 'undifferentiated' sinus epithelium are striking. The 'epidermal' epithelium develops *pari passu* with the perineal epidermis and is presumably an ectodermal derivative, representing the internal zone of a sexual skin area. The 'undifferentiated' sinus epithelium persists until the 140 mm. stage and never bears any histological resemblance to the 'epidermal' epithelium. This suggests that the 'undifferentiated' epithelium is not of ectodermal origin, and the sharp junction between it and the 'epidermal' epithelium implies that there is no mixture of ectoderm and endoderm in this region. If there were, as Zuckerman (1940) suggested generally and as Burns (1942) described in the opossum, an ingrowth of ectoderm in the caudal part of the human sinus, and an impression of ectodermal characters on the rest of the sinus epithelium, one would surely expect

more similarity between the 'undifferentiated' sinus epithelium and the 'epidermal' epithelium.

At the 180 mm. stage the sinus is occupied by the 'vaginal' and 'glandular' types of epithelium. Hunter (1930) described a downgrowth of vaginal epithelium into the vestibule after the 200 mm. stage, but it is impossible to decide whether the 'vaginal' type of vestibular epithelium does arise in this manner or as a differentiation *in situ* of the 'undifferentiated' sinus epithelium. The 'glandular' type of epithelium must, apparently, be derived from the 'undifferentiated' sinus epithelium. Since the junction between 'vaginal' and 'glandular' types of epithelium is usually fairly gradual, it is probably more likely that the 'vaginal' type of vestibular epithelium is also derived from the earlier 'undifferentiated' sinus epithelium.

Both 'vaginal' and 'glandular' types of epithelium are clearly different from the 'epidermal' epithelium, and the junction between the 'vaginal' type of epithelium and the 'epidermal' epithelium is always clear cut. Neither of the two epithelial types which arise in the urogenital sinus therefore bears any great resemblance to the developing epidermis of the sexual skin area. Moreover, since the later stages of development are probably influenced by a high level of oestrogenic stimulation (Fraenkel & Papanicolou, 1938), it would seem that the response to oestrogenic stimulation of the epithelia derived from the urogenital sinus is not identical with the response of the sexual skin area. The response of the 'vaginal' type of vestibular epithelium, like that of the vaginal epithelium itself, is associated with the accumulation of large amounts of glycogen and the formation of 'intercellular walls'. The response of the 'epidermal' epithelium and the rest of the sexual skin is associated with a gradual decrease in glycogen content and the formation of a thick superficial layer of keratin. The response of the 'glandular' epithelium, if it too is sensitive to oestrogenic stimulation, is even more remote from that of the sexual skin area. There seems no good reason, therefore, to support Zuckerman's view that there is an ingrowth of ectodermal epithelium into the urogenital sinus, or to oppose the classical view that the mucocutaneous junction of the vestibule is a junction between two epithelia of differing origins.

However, while this indicates that the evidence which Zuckerman adduced in support of his hypothesis is not applicable to the development of the human sinus, it does not necessarily mean that the hypothesis is irrelevant. A discussion of the human sinus epithelium cannot ignore the small area of 'differentiated' epithelium in the region of the Müllerian tubercle, which gives rise, by its proliferation, to the epithelial lining of the entire vagina (Bulmer, 1957). The vaginal epithelium responds to oestrogenic stimulation by a stratified squamous cell proliferation. If Hunter's view on the downgrowth of vaginal epithelium into the vestibule is correct, then the 'vaginal' type of epithelium is also derived from the small area of 'differentiated' sinus epithelium. Even if the 'vaginal' type of epithelium does arise *in situ* from the 'undifferentiated' sinus epithelium, it may be that this differentiation is induced by the proximity of the vaginal epithelium itself. It is possible, therefore, that the 'differentiated' epithelium is ultimately responsible for the formation of all the sinus epithelium which is capable of a stratified squamous cell proliferation in response to oestrogenic stimulation. It is, then, the origin of the small area of 'differentiated' sinus epithelium in the region of the Müllerian tubercle which is

pertinent to the origin of the oestrogen-sensitive sinus epithelium, rather than the question of a possible ingrowth of oestrogen-sensitive ectoderm from below.

Because of the separation of the area of 'differentiated' epithelium from the 'epidermal' epithelium between the 28 and 140 mm. stages, it seems unlikely that the 'differentiated' epithelium can arise as an ectodermal ingrowth. Further, because of its position in the dorsal wall of the sinus, it is unlikely, if the conventional description of the early development of the sinus is correct (Felix, 1912), that it could incorporate a remnant of the cloacal membrane or the primitive streak, which Zuckerman suggested as possible sources of the oestrogen-sensitive sinus epithelium. However, the small area of 'differentiated' epithelium does represent a centre of proliferation, and probably of organization. While it is unlikely, in view of the findings of the present investigation, that it is ontogenetically an ectodermal derivative, the account by Burns of the development of the oestrogen-sensitive sinus epithelium in the opossum suggests that there may be a phylogenetic relation with ectoderm. A further point in this connexion is the resemblance, both histological and also, according to Wislocki, Fawcett & Dempsey (1951), histochemical, between the vaginal epithelium and certain other stratified squamous mucous membranes which are generally accepted to be of ectodermal origin.

The small area of 'differentiated' epithelium in the urogenital sinus, which plays such an important part in female development, seems also to occur in the male embryo (Glenister, 1958), where it occupies the region around the lower ends of the Müllerian and Wolffian ducts. It is interesting that it is this area which Zuckerman described as the oestrogen-sensitive zone of the urogenital sinus in the human male. It is possible, therefore, that this oestrogen-sensitive epithelium corresponds with the oestrogen-sensitive vaginal epithelium and 'vaginal' type of vestibular epithelium in the female. The epithelium of the remainder of the male sinus, which is apparently insensitive to oestrogens, may then correspond with the 'glandular' type of vestibular epithelium in the female, though the decrease in the latter epithelium after the time of birth suggests that it may be dependent on hormonal stimulation.

The peculiar differentiation of the sinus epithelium over the region of the Müllerian tubercle has been noticed by many previous workers, and this subject has been briefly discussed elsewhere (Bulmer, 1957). Many authorities (Vilas, 1932; Kempermann, 1931; Meyer, 1934-8) have considered that the pale-staining cells of the inner zone of this epithelium represent an ingrowth from the lower ends of the Wolffian ducts, though Kempermann (1935) considered that the Wolffian cells soon degenerated and played no part in the formation of the vagina. Whether the pale-staining internal cells are really of Wolffian origin or whether they arise from the more deeply staining basal cells appears to be another problem which cannot be solved by histological studies. However, it is likely that the vaginal primordium develops principally from the darkly staining basal cells and that the Wolffian ingrowth, if it does occur, is only transitory.

It is remarkable that similar histological findings have not been recorded in other mammals. In the sheep, for instance, the entire sinus is lined by a pale-staining stratified epithelium. By the proliferation of this epithelium in the region of the Müllerian tubercle the primordium of the lower vaginal segment is formed (Bulmer, 1956). Thus, while there is no clear histological distinction between the epithelium

over the Müllerian tubercle and that of the rest of the urogenital sinus, as there is in the human embryo, the epithelium over the Müllerian tubercle has a proliferative capacity similar to that which occurs in the human.

It is difficult to visualize what the significance of these differences may be. However, it is clear that the sinus epithelium in the region of the Müllerian tubercle has the capacity to proliferate and form an epithelium which gives a stratified squamous response to oestrogenic stimulation, though in human foetal material this response is not, apparently, identical with the stratified squamous response of the epidermis of the sexual skin area. In the human female it is possible that the only epithelium derived from the urogenital sinus which is capable of a stratified squamous response is the epithelium which has arisen from the small 'differentiated' area originally confined to the Müllerian tubercle. There is no evidence to indicate that this is so in the sheep, and from the work of Burns it would seem not to be true in the opossum. However, in both these forms (Baxter, 1935 for the opossum), as well as in many other mammals (for bibliography see Bulmer, 1955), the sinus epithelium in the region of the Müllerian tubercle does form a proliferative zone which makes a contribution to the vagina.

SUMMARY

1. The epithelium of the urogenital sinus is described in female human foetuses and a female infant.

2. There is no resemblance at any stage between the epithelium of the 'sexual skin' area and the epithelia of the urogenital sinus.

3. It is possible that all the sinus derivatives which respond to oestrogenic stimulation by a stratified squamous proliferation arise from, or under the influence of, a small area of 'differentiated' sinus epithelium originally confined to the region of the Müllerian tubercle.

4. The origin of this area of 'differentiated' epithelium, its comparative embryology and possible homology in male development are briefly discussed.

My thanks are due to Prof. R. D. Lockhart for his advice and criticism on the preparation of this paper, and to Prof. J. D. Boyd for making his collection of human embryos and foetuses available to me. I am also indebted to Mr J. A. F. Fozzard and Mr A. Cain for the photographs.

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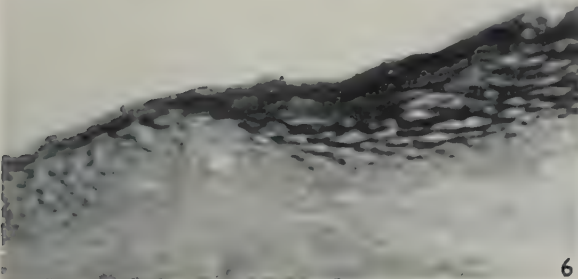
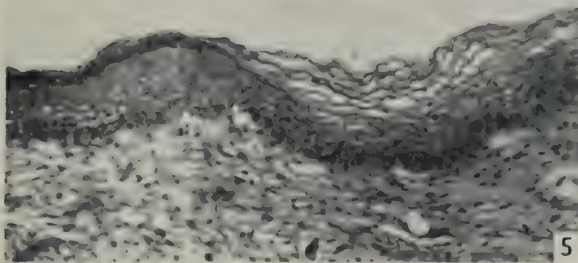
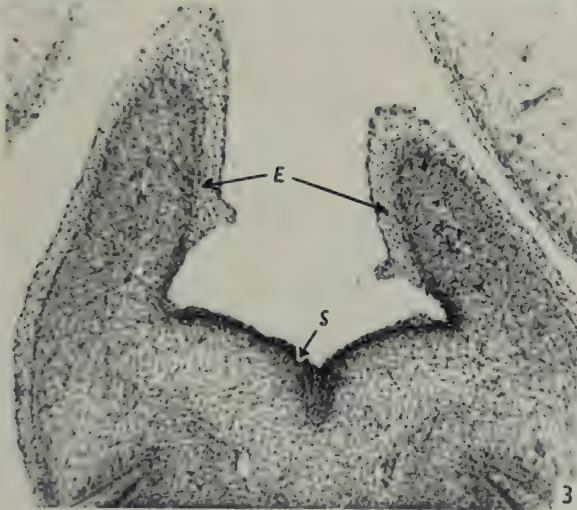
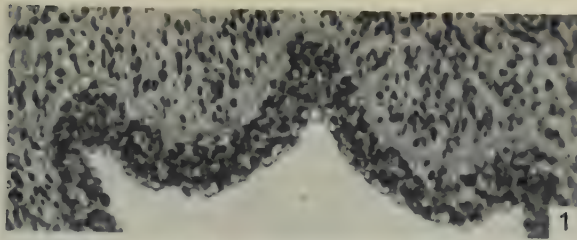
EXPLANATION OF PLATES

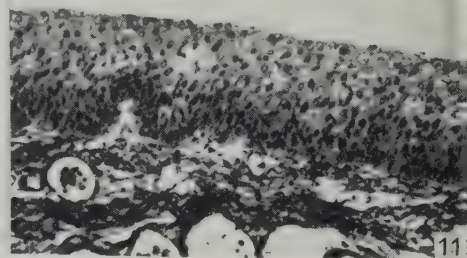
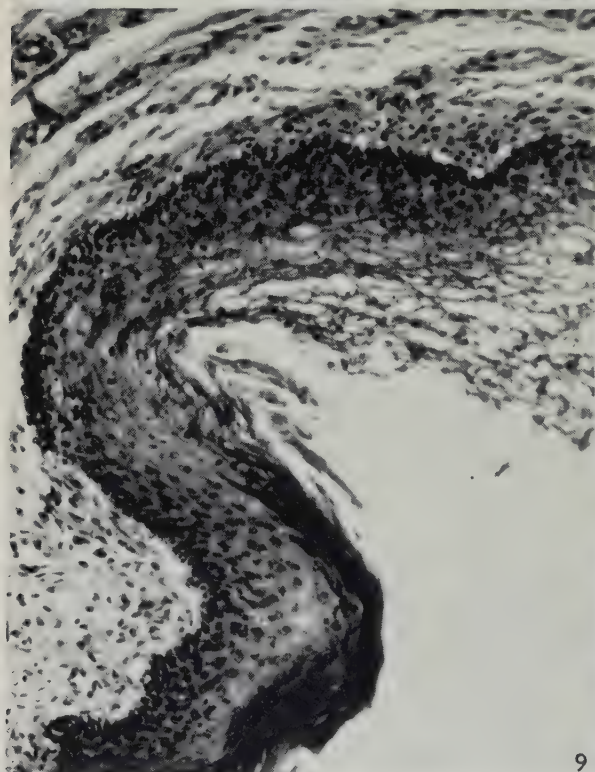
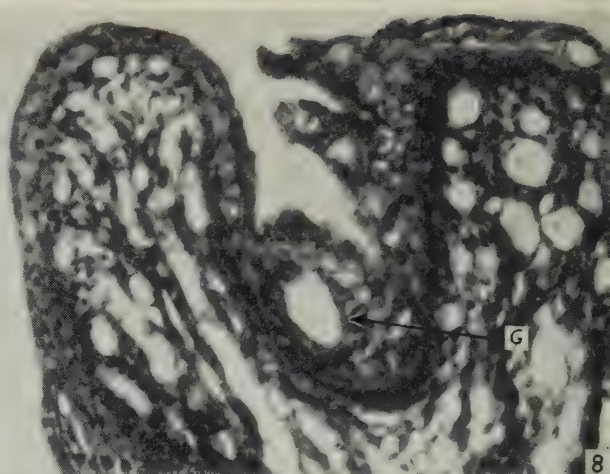
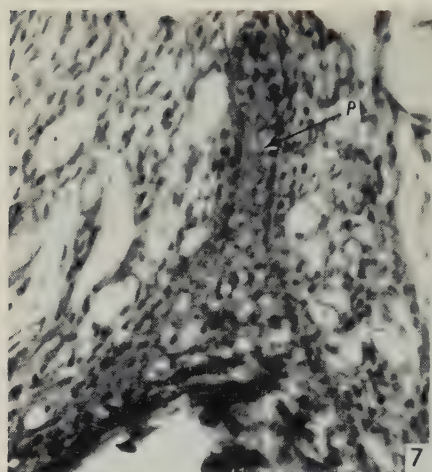
PLATE 1

- Fig. 1. The darkly staining epithelium of the ventral wall of the pars pelvina in the 28 mm. embryo. $\times 215$.
- Fig. 2. The phallus and urethral plate in the 28 mm. embryo. At the tip of the phallus the rather pale-staining epithelium of the urethral plate merges indistinguishably with the epidermis. $\times 60$.
- Fig. 3. Coronal section through the ventral wall of the pars phallica in the 65 mm. foetus. The darkly staining epithelium of the urethral plate is flanked on either side by the thin darkly staining epithelium (*S*) of the 'secondary urethral groove'. This is sharply distinct from the thicker pale-staining 'epidermal' epithelium (*E*) over the inner aspects of the urethral folds. $\times 60$.
- Fig. 4. Transverse section through the caudal part of the pars phallica in a 16½-week foetus. The darkly staining 'undifferentiated' sinus epithelium (*S*), over the dorsal and ventral walls, is sharply distinct from the 'epidermal' epithelium (*E*) laterally, over the inner aspects of the urethral folds. $\times 23$.
- Fig. 5. Junction between the 'epidermal' epithelium (left) and the 'vaginal' type of vestibular epithelium in the 180 mm. foetus. $\times 105$.
- Fig. 6. A similar section to that shown in fig. 5, stained by the PAS technique. $\times 105$.

PLATE 2

- Fig. 7. The ventral aspect of the glans in the 180 mm. foetus (PAS and haematoxylin). The tiny epithelial invagination (*P*) appears to be a remnant of the urethral plate. $\times 200$.
- Fig. 8. The 'glandular' type of vestibular epithelium in the 180 mm. foetus. An intra-epithelial gland is shown at *G*. $\times 240$.
- Fig. 9. The junction of the 'epidermal' epithelium (below) and the 'vaginal' type of vestibular epithelium in the 375 mm. foetus. $\times 115$.
- Fig. 10. The 'glandular' type of epithelium in the 375 mm. foetus, stained by the PAS technique. The walls of the alveolar spaces give a marked reaction. $\times 100$.
- Fig. 11. The 'vaginal' type of vestibular epithelium in the 1-month infant. $\times 120$.





ANATOMY OF THE PELVIS IN THE RABBIT KIDNEY

By H. L. SHEEHAN AND J. C. DAVIS

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In the course of a study of experimental hydronephrosis (Sheehan & Davis, 1959), it became clear that a detailed knowledge of the anatomy of the pelvis in the rabbit kidney was fundamental to the understanding of the lesions. We have not been able to find a comprehensive description of the anatomy of the unipyramidal kidney, though individual aspects have been recorded in the literature.

The shape of the medulla and intermediate zone

The medulla of the rabbit kidney is shaped like a segment of an orange, with the narrow ends curled round at the upper and lower poles of the kidney so that they point towards the hilum. The pyramid proper is a projection of the central part of the medulla, and has the form of a cone considerably flattened from front to back (i.e. ventro-dorsally).

There is a well-defined intermediate zone, which forms a broad band between the cortex and medulla. The outer half of this band contains the spiral segments of the proximal tubules; the inner half consists mainly of broad ascending limbs of Henle's loops. In that part of the kidney which lies directly beneath the base of the pyramid, the tubules of the intermediate zone run radially between the cortex and the medulla. On the other hand, the tubules from the ventral and dorsal surfaces of the kidney have to run in a transverse arc around the outside of the pelvis in order to reach the base of the medulla. Owing to the particular arrangement of the large vessels and the shape of the pelvis in this region, these transversely-running portions of the intermediate zone are massed into about six main 'peripelvic columns' on each side of the pelvis. These columns project inwards as blunt ridges which run transversely around the pelvis to the fornix, where they turn sharply round to continue into the related part of the medullary pyramid. At this bend they occupy the concavity of the corresponding part of the free semilunar edges of the pelvic septum.

The pelvic septa

The hilum of the kidney leads into a short 'hilar tunnel', about 3 mm. long, whose walls consist of cortex. This hilar tunnel transmits the ureter, vessels and nerves; these are embedded in fat and fibrous tissue, which is attached to the cortex along the whole length of the tunnel. The mass of tissue, with the main renal artery and vein which it contains, divides at the inner end of the hilar tunnel into a ventral and a dorsal portion, each of which then spreads out in the renal sinus to form a large connective tissue septum (Pl. 1, fig. 1), one lying ventral to the medullary pyramid and the other dorsal to it. They are separated from the medulla by the corresponding halves of the lumen of the primary pelvis.

Each septum has a scaffolding of about five spokes which radiate out around the

pelvis from the root of the septum at the hilum. These consist of the five main interlobar branches of the renal artery and vein, and are referred to here as the 'vascular bundles'. At their tips they are inserted into the substance of the kidney in the longitudinal groove (the 'fornix') alongside the base of the pyramid, but between these attachments the outer margin of the septum is free and has a semilunar edge.

At its base near the hilum, the septum is continuous with the interstitial tissue in the hilar tunnel, and so is attached to the cortex at this site. From here it runs straight out towards the fornix. The inner face and most of the outer face of the septum lie free in the pelvic cavity, but on the outer face, along the line of each of the five vascular bundles, a 'subsidiary septum' runs out at right angles to the main septum. Each of these subsidiary septa is attached to the kidney tissue like a mesentery, with a linear insertion along the groove between two neighbouring peripelvic columns (Pl. 1, fig. 2).

At intervals, the vessels of the vascular bundles in the pelvic septum proper give off branches, which run radially outwards in the subsidiary septa and enter the renal parenchyma, where they become arcuate vessels of the ventral and dorsal parts of the kidney. The distal end of each of the vascular bundles finally reaches the inner surface of the kidney tissue in the fornix; here the vessels plunge into the intermediate zone and arch over the base of the pyramid as the arcuate vessels in the convex part of the kidney.

These arcuate vessels carry with them a heavy band of tissue into the intermediate zone. This 'arcuate fibrous band' consists mainly of collagen fibres with some fine elastic fibrils and also, near the fornices, numerous smooth muscle fibres which appear to correspond to the levator anguli fornicis muscle of the human kidney. In the substance of the kidney, the arcuate band lies on the medullary side of the arcuate vessels, and is connected to them by a thinner mass of fibrous tissue.

The smooth muscle layer of the ureter is continued upwards the epithelium on the entire inner face of the septum as a large sheet. It consists of a thick internal layer running longitudinally along the septum, and a thin outer layer which runs from the hilum towards the fornix. Along the lateral (fornical) edge of the septum, at the points where the vessels enter the parenchyma, the septal muscle becomes continuous with the levator anguli fornicis. The outer face of the septum has merely a thin layer of connective tissue beneath the epithelium. Between this fibrous layer and the muscle layer, the main substance of the septum consists almost entirely of fat, in which lie the vessels, nerves and lymphatics. This fat often forms a very prominent feature of the septum, so much so that the entire septal tissue is commonly referred to under the broad generalization of 'pelvic fat'.

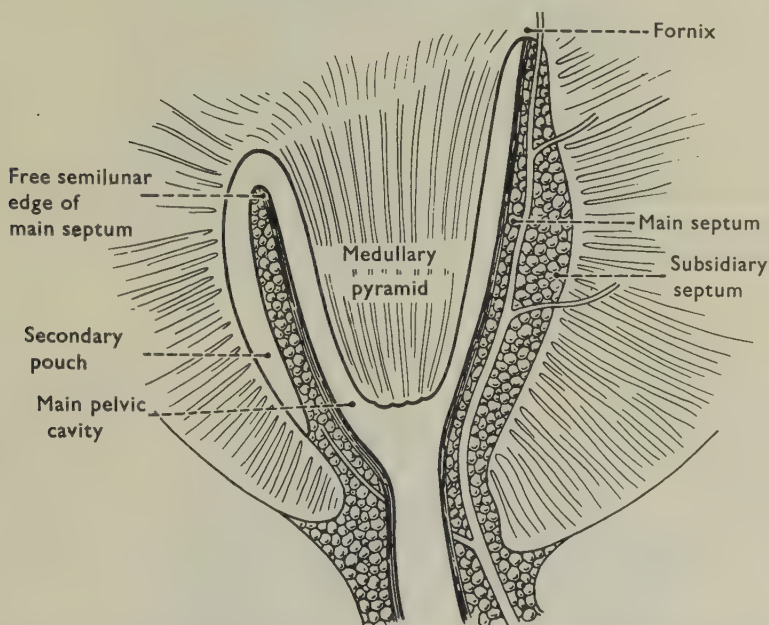
The cavity of the pelvis

The word 'pelvis' is often used in a broad sense to include all the structures of the renal sinus, but in the present context it is employed to mean only the epithelium-lined space which is formed by the expanded upper end of the ureter.

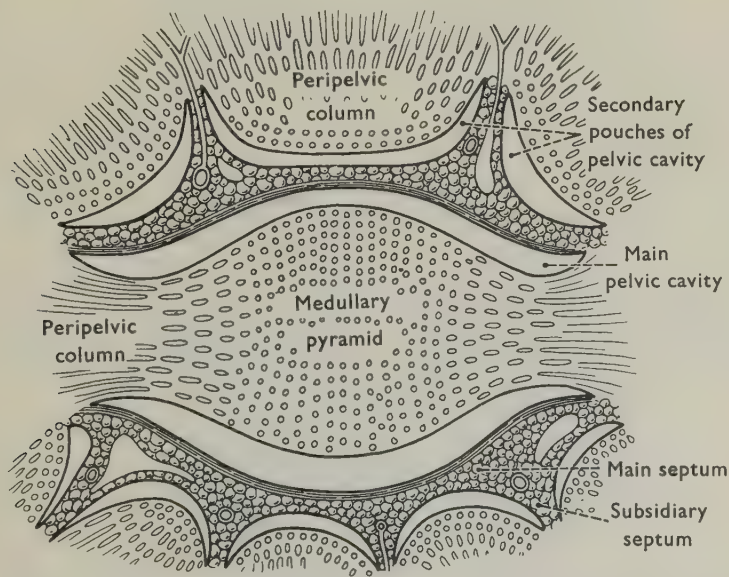
After the ureter has entered the kidney through the hilar tunnel, its cavity spreads out on each side between the medulla and the inner surface of the septum. The resultant two large flat spaces may be regarded as the primary parts of the pelvis. The pelvis then protrudes over the free semilunar edges of the septum and

turns back on itself so that it covers the outer face of the septum, right down to the point at which the septum is attached at the inner end of the hilar tunnel (Text-fig. 1.)

This retroflexed part of the pelvis is not a continuous cavity; it is divided up by the subsidiary septa into a series of six separate 'secondary pouches' (Pl. 1, fig. 3).



Text-fig. 1. Transverse section of kidney at hilum. On the left, the main septum is cut through its free semilunar edge; on the right it is cut along one of the vascular bundles and the subsidiary septum.



Text-fig. 2. Longitudinal section of kidney in plane midway between hilum and convex border.

These communicate with each other at their origins from the fornical part of the pelvis. The external surface of each secondary pouch is deeply concave in its long axis (i.e. running in the direction from the hilum towards the fornix), the hollow being occupied by one of the peripelvic columns. The lateral edges of the pelvic pouches project deeply into the grooves in the angle between the peripelvic columns and the insertion of the subsidiary septa (Text-fig. 2).

The transitional epithelium of the ureter covers the inner face of the septum and continues over the free semilunar edge to cover about the upper one quarter of the outer face of the septum. Here it changes to a columnar epithelium which lines all the remainder of the secondary pouch and covers the medullary pyramid.

Sometimes a thin layer of parenchyma, about 3 or 4 tubules deep, extends from the cortex near the hilar tunnel under the outer face of the main septum for about one third of the distance towards the free semilunar edge.

SUMMARY

In the unipyramidal kidney of the rabbit, the pelvic tissues form two septa, which enclose the medullary pyramid. The pelvic cavity extends up between the septa and the pyramid to the fornix and then turns outwards to form a series of secondary pouches on the outer surface of the pelvic septa. The blood vessels run in five main vascular bundles radiating out in the septa. At intervals these vessels give off branches to the renal parenchyma, passing in subsidiary septa which reach the substance of the kidney between the secondary pouches.

REFERENCE

SHEEHAN, H. L. & DAVIS, J. C. (1959). Experimental hydronephrosis. *Arch. Path.* 68, 185-225.

EXPLANATION OF PLATE

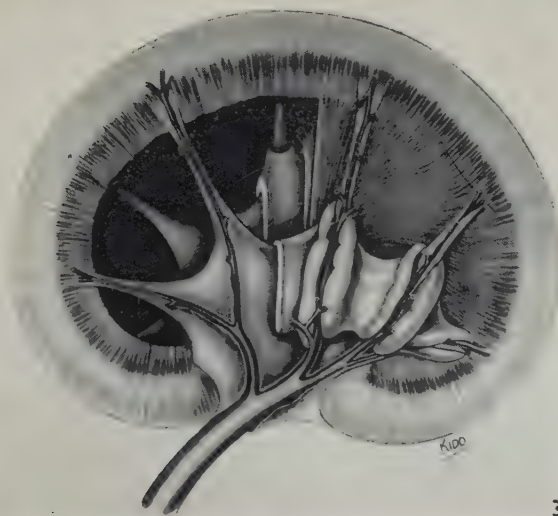
- Fig. 1. Kidney dissected to show the two pelvic septa with their arteries. On the left, the medulla and pyramid have been removed to show the pelvic surface of the deeper septum. The subsidiary septa of the front septum have been cut off at their origin from the vascular bundles.
- Fig. 2. The deeper one-third of the kidney after removal of the medulla and pyramid and of the main septum on that side. The subsidiary septa are shown in cross-section near their attachment to the underlying kidney tissue. The wedges of parenchyma projecting forward between them are the peripelvic columns; these are cut across at their outer part where they turn sharply to enter the medulla.
- Fig. 3. A diagram similar to fig. 1 but with the addition of the pelvis on the right. The pelvic cavity has been filled with an injection material, so that it appears here as a solid mass. At the back, the main part of the pelvis (distended by injection mass) lies between the medullary pyramid and the septum, and turns back over the free semilunar edge of the septum. In front of the front septum, the pelvis extends down as the secondary pouches whose centres are deeply indented by the peripelvic columns.



1



2



3

FACTORS AFFECTING THE ELASTICITY OF BONE

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INTRODUCTION

When any substance is subjected to stress it is deformed by that stress. If it recovers its original dimensions when the stress ceases it is termed elastic, whereas if the deformation persists in whole or in part it is termed inelastic or plastic. For any elastic substance there is an upper limit of stress, namely the elastic limit, beyond which the property of elasticity is lost.

The extent of the deformation caused by a given intensity of stress varies in different elastic substances. In the case of tensile stress the extensibility is usually expressed inversely by the constant known as Young's modulus (E). Thus under a given tensile stress a piece of rubber will extend more than a piece of steel of the same dimensions and therefore the value of Young's modulus for rubber is lower than that for steel.

Although Young's modulus is thus defined in terms of tensile strength, it is also closely related to the flexibility of a body when it is subjected to a bending stress, so that a low value of Young's modulus is associated with comparatively flexible substances and vice versa. For this reason the modulus is frequently used as an inverse index of both the extensibility and the flexibility of materials, and its value for bone from various sources has been determined by many authors.

In the course of an investigation of the relationship between the elastic properties of bone and its microscopic structure (Smith & Walmsley, 1957; Walmsley & Smith, 1957) it became apparent that the standard methods for determining Young's modulus for this tissue were unsatisfactory. The present communication gives an account of the factors which influence the elastic properties of bone and indicates standards for the determination of Young's modulus which would permit the elastic properties of different bone specimens to be accurately compared.

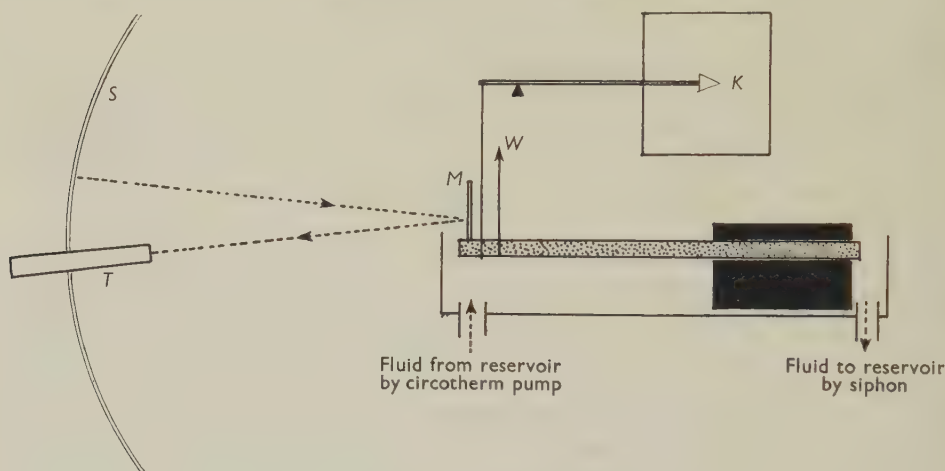
MATERIALS AND METHODS

The observations recorded in this paper were made on bone from the human tibia, the radius of the horse, the metacarpus of the ox, the metacarpus of the sheep and the femur of the dog. The shaft of the bone to be examined was cut into longitudinal rods on a bandsaw and each rod was then prepared to a uniform rectangular cross-section by planing. It was considered that any tissue which had been damaged by heat during the preliminary sawing was removed by the subsequent planing. The final size of the rod varied with its source, but the average length was about 3 in. and the average cross-section about 0.02 sq. in.

In the preliminary stages of the investigation the elastic properties of the test pieces were assessed by measuring the deformations caused by bending stress, axial tensile stress, and axial compression stress.

Bending stress (cantilever method)

The essentials of the method are shown in Text-fig 1. A shallow groove was filed transversely across one of the wider surfaces of the bone rod about half an inch from one end, and the other end was then fixed horizontally in a clamp so that the groove lay on the lower surface. A cord loop, which passed under the rod so that it lodged in the groove, was then attached directly above to one of the equal arms of a balanced beam. When a weight (W) was applied to the other end of the beam, the rod bent so that the free end was carried upwards in relation to that fixed in the clamp. Moreover, as the rod bent its upper surface became inclined to the horizontal so that at any given deflexion the inclination increased in value from the clamp as far as the groove, beyond which point it was constant.



Text-fig. 1. Determination of Young's modulus by the cantilever method.
The bone rod is stippled.

In some experiments the upward displacement of the free end of the rod was recorded on a kymograph (K) as indicated in Text-fig. 1. From such a recording the value of Young's modulus (E) could be calculated, but because the method is less accurate than that described below, kymographic recordings were made only when a continuous record of the deflexion caused by a prolonged load was required.

More frequently the value of Young's modulus was determined by measuring the angulation to the horizontal of the upper surface of the rod at or beyond the groove. For this purpose a small plane mirror (M) was attached to the rod between the groove and the free end. A curved scale (S) with a radius of 12 ft., calibrated in $\frac{1}{10}$ in., was placed so that its centre was coincident with the mirror, and a telescope (T), with crossed wires in the eyepiece, was arranged so that the scale could be viewed by reflexion from the mirror. Any angulation of the rod caused an equal angulation of the mirror in relation to its original position and consequently a change in the scale reading as seen through the telescope.

It is appreciated that whatever the method used to fix the rod, the fixation can never be absolute. In practice therefore two bone rods of identical measurements

were always fixed side by side in the clamp and each carried a mirror so arranged that two images of the scale were seen side by side in the telescope simultaneously. In this way any distortion in the clamping mechanism was shown by a scale deflexion in the control mirror, and this was subtracted from that in the test piece mirror to give the true scale deflexion. If this corrected scale deflexion is denoted by X , then Young's modulus is

$$E = \frac{1728WL^2}{AB^3X} \text{ lb./sq.in.}, \quad (1)$$

where W is the applied load in pounds, L the length of the bone rod from the clamp to the groove, A its width and B its depth, all in inches.

It is recognized that equation (1) involves an approximation as it ignores the effect of shear stress during bending. It may be shown that the percentage error associated with the use of this approximation is $30EB^2/NL^2$, where N is the modulus of rigidity. Although no observations have been made during this investigation, the approximate value of N for bone may be calculated from the results of torsion experiments carried out on preserved human femora by Carothers, Smith & Calabrisi (1949). If the shaft of the adult femur is regarded as being approximately cylindrical with outer and inner cortical diameters of 1.15 and 0.77 in. respectively, it can be calculated that the modulus of rigidity has a value of 8.0×10^5 lb./sq.in. The value of E is of the order of 2.0×10^6 lb./sq.in. and therefore the percentage error involved in the use of equation (1) is 0.25 %. Admittedly this evaluation of the modulus of rigidity (N) is approximate, but it is sufficient to show that the error involved in the use of equation (1) for the calculation of Young's modulus is probably insignificant.

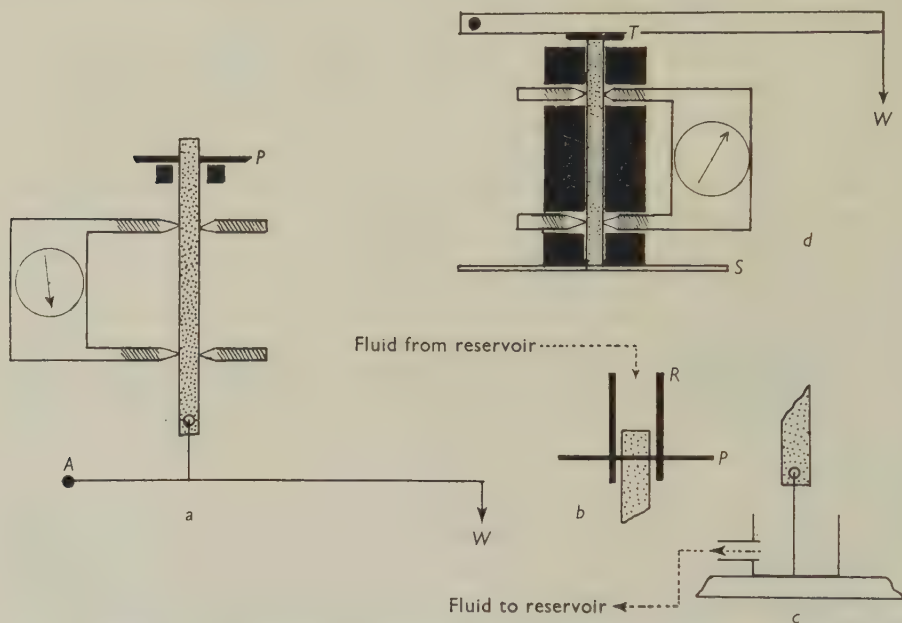
It was considered appropriate during the investigation to evaluate Young's modulus with the bone immersed in fluid and at various temperatures. This was achieved by enclosing the clamp and the bone rod in a fluid bath of such a depth that the mirror projected above the sides. Fluid at any required temperature was then driven by a circotherm pump from a large fluid reservoir to the bath and returned by siphon.

Axial tensile stress

The method is illustrated in Text-fig. 2*a*. The upper end of the test piece was transfixed by a steel pin (P) which rested on two metal supports. The lower end was attached to a beam 4 in. from its axis (A) and the load (W) was applied 28 in. farther along the beam so that the force exerted on the rod was $8W$. The extension of the central 2 in. of the rod was measured correct to $\frac{1}{20,000}$ in. by a Lyndley extensometer. If this extension is denoted by (T) then the value of Young's modulus is $E = 16W/ABT$ lb./sq.in. Here again it was found necessary to determine E with the bone in a fluid medium and at various temperatures. This was done by fitting a rubber tube (R) loosely over the upper end of the bone rod so that both the tube and the rod were transfixed by the steel pin (P) (Text-fig. 2*b*). Fluid at the required temperature was driven by a circotherm pump from a reservoir on to the upper end of the rod. Thereafter it flowed down the rod as a continuous film and collected in a receiver which surrounded the attachment of the rod to the beam (Text-fig. 2*c*). From here the fluid was returned to the reservoir by siphon.

Axial compression stress

The method is illustrated in Text-fig. 2*d*. The bone rod rested on a firm metal support (*S*) and its upper end was in contact with a metal plate (*T*) fixed to the under-surface of a beam 4 in. from its axis. The load (*W*) was applied at a point 28 in. farther along the beam so that the force exerted on the rod was $8W$. The contraction of the central 2 in. of the rod (*C*) was measured by the Lyndley extensometer and from this measurement the value of Young's modulus was calculated from the equation $E = 16W/ABC$.



Text-fig. 2. Determination of Young's modulus under axial tensile stress (*a*, *b* and *c*) and under axial compression stress (*d*).

Axial compression of a thin rod almost inevitably tends to cause bending as well as contraction, but this tendency was overcome by the standard method of enclosing the rod in a close-fitting metal collar. This collar is shown in black in Text-fig. 2*d* and it will be noted, first, that it was about $\frac{1}{16}$ in. shorter than the bone rod, and secondly, that it had windows which permitted the attachment of the extensometer screws. As in the other two methods, it would have been desirable to carry out some of the observations with the bone immersed in a fluid medium but no satisfactory method for this has been achieved.

OBSERVATIONS

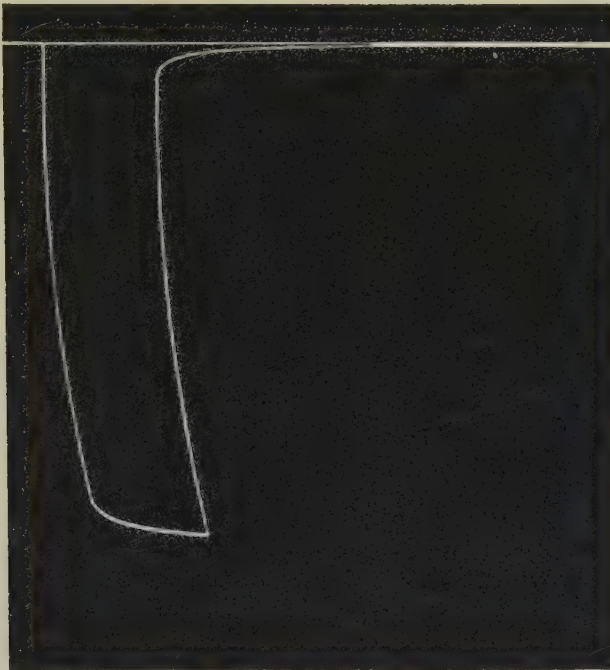
Comparison of the effects of tensile and compression stresses

The axial compression method was used for one purpose only, namely to confirm that Young's modulus for bone had the same value when it was determined by the axial tensile method (E_T) and the axial compression method (E_c). Although such

a relationship exists in most materials it had to be confirmed because it is assumed in the calculations necessary to determine Young's modulus by the cantilever method. Because of the technical difficulty of measuring the compression of bone in a fluid medium, the comparison was necessarily made with the test pieces in air, but with this reservation it was found that in all the specimens examined, the value of E_c was less than 3 % greater than that of E_T . It is considered that this difference was due to the slight friction between the rod and the shield which is inherent in the axial compression method.

The effect of stress duration on the deformation of bone

The behaviour of bone under stress is illustrated by the kymogram in Text-fig. 3, which shows the deflexion of the free end of a bone rod arranged as a cantilever when it is subjected to a prolonged bending stress, and by the graph in Text-fig. 4, which shows the elongation of a bone rod subjected to a prolonged tensile stress.



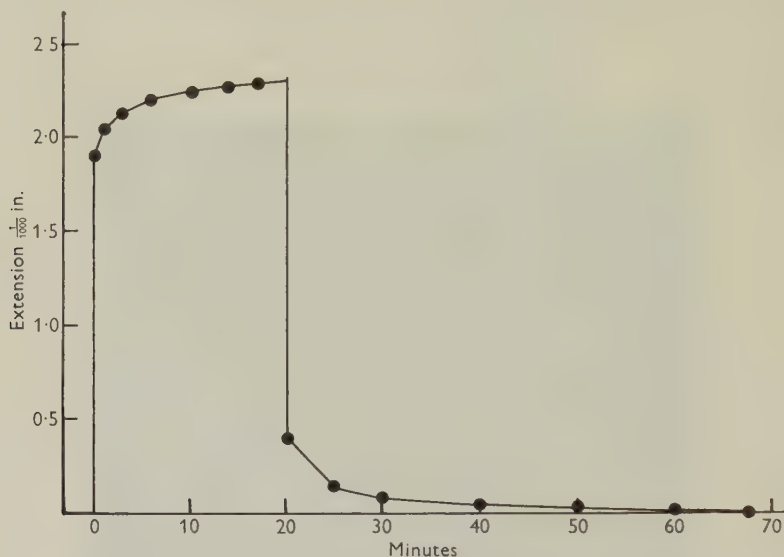
Text-fig. 3. Kymogram showing the deflexion and recoil of the free end of a bone rod subjected to a bending stress of 4 min. duration.

It is apparent that in both instances there was an immediate deformation, coincident with the application of the stress, and that thereafter the deformation continued to increase asymptotically while the stress remained constant. When the stress ceased an immediate incomplete recoil was followed by an asymptotic approach to zero which was similar though more prolonged.

This is the phenomenon of elastic after-effect. Because of this phenomenon the deformation of bone caused by a stress below the elastic limit depends on the duration of the stress and the difference between the immediate and the ultimate

deformations may be as much as 10%. Moreover, because Young's modulus is always calculated from an observed deformation it is evident that this constant is necessarily related to stress duration in a similar manner.

Elastic after-effect is not peculiar to bone. On the contrary it is common to all the supporting tissues of the body, namely bone, cartilage (Bär, 1926; Hirsch, 1944) and the intervertebral discs (Virgin, 1951), and it is observed also in the stress/strain relationships of many inanimate materials such as glass and Perspex. According to Poynting & Thomson (1902) the magnitude of the phenomenon is dependent on the constitution of the material, being negligible in homogeneous bodies such as crystals and large in those materials whose fine structure is heterogeneous, such as glass.



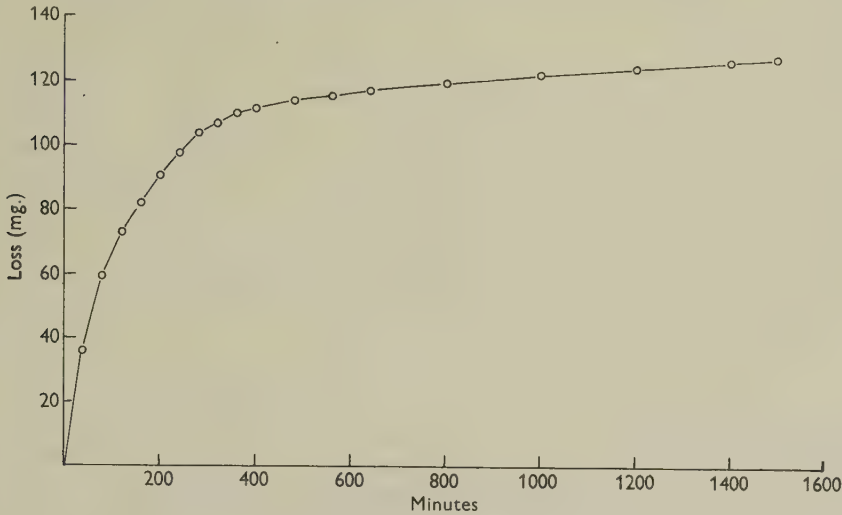
Text-fig. 4. The extension and recoil of a bone rod subjected to a tensile stress of 20 min. duration.

This fact may serve in part as an explanation of the elastic after-effect in bone, but it is possible that another process is also involved. It has been proposed by Ingelmark & Ekholm (1948) that the elastic after-effect noted during the compression of cartilage is the result of a dual mechanism in both compression and recoil. They suggested that both processes were due in part to true elasticity and in part to a migration of fluid between the cartilage on the one hand and the synovial fluid and the vessels of the marrow cavity on the other. It has long been recognized that similar migration of fluid is involved in the compression and recoil of the intervertebral discs and it is possible therefore that the same mechanism is partially responsible for the after-effect in bone.

The effect of water content on the elastic properties of bone

Bone, like any other tissue, begins to lose fluid by evaporation as soon as it is removed from the body. The rate of evaporation in milligrammes from a block of bone having a surface area of 20.2 cm.² and a weight, 11 min. after removal from the

body, of 3.9 g. is shown in Text-fig. 5. As would be expected, the rate decreased progressively, so that 2.7 % by weight was lost after 5 hr. and 3.3 % after 24 hr. This loss continues until equilibrium with the atmosphere is established, and although its duration varies with the dimensions of the bone block and the humidity of the atmosphere the loss is practically complete within a week.

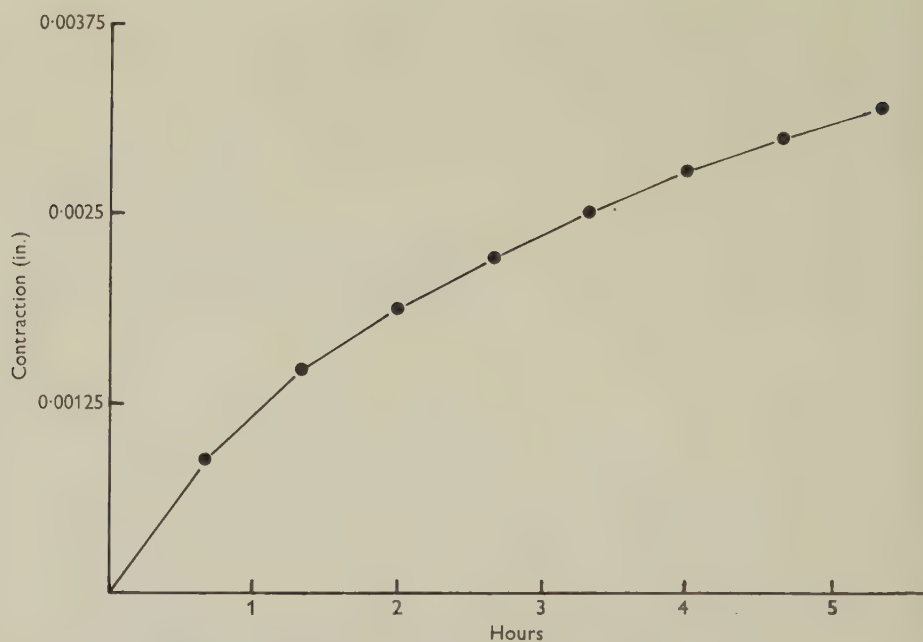


Text-fig. 5. Loss in weight due to evaporation from a bone rod.

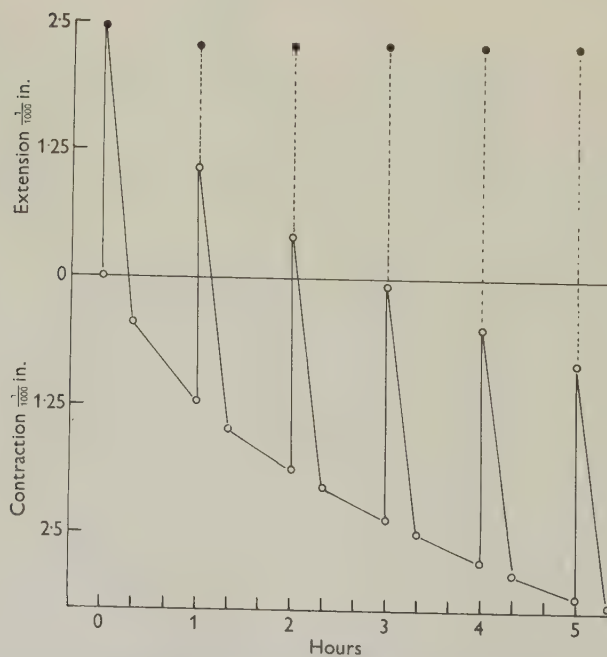
However, a loss of weight is not the only change which occurs during the process of evaporation; there is also a progressive diminution in the size of the test piece. Text-fig. 6 shows the contraction occurring in a bone rod between 10 min. and 5 hr. after its removal from the body. Even after 5 hr. the 2 in. between the extensometer screws had only decreased by 0.003 in. and it may appear at first sight that a contraction of this order is of no great significance. However, when it is appreciated that the extension of one of the bone rods used in this investigation under an axial tensile load of 70 lb. is only of the order of 0.002 in., it becomes apparent that the contraction associated with evaporation is an important factor.

If at any time during the process of evaporation the bone rod is placed in physiological saline its length increases and eventually exceeds the original measurement made 10 min. after its removal from the body. The balance of the increase is presumably a reversal of the contraction occurring during the 10 min. necessary for the removal and preparation of the rod, and although it is impossible to prove that the final length of the rod in saline is equal to its length within the body, the approximation is considered to be fairly close.

To assess the effect of evaporation from bone on the value of Young's modulus a bone rod was prepared and immediately arranged for the application of axial tensile stress in fluid (Text-fig. 2a-c). An hour later, when it was presumed that the fluid loss due to evaporation occurring during removal and preparation of the rod had been corrected, a load was applied and the extension which had occurred after 2 min. was measured (Text-fig. 7). The fluid circulation was then stopped so that the rod began to dry. Thereafter the contraction of the rod was observed over a period



Text-fig. 6. Contraction of a bone rod accompanying evaporation from its surface.



Text-fig. 7. Graph showing the contraction of a bone rod due to evaporation from its surface, and the extensions produced by the application of the same load at hourly intervals during the period of evaporation.

of 5 hr. and the extension caused by the same load was measured at hourly intervals. It was found that, after allowing for the contraction due to evaporation, the extensibility of the bone was 7 % greater at the start of the experiment than after 1 hr. Thereafter the extensibility remained constant. Young's modulus is inversely proportional to the extensibility and therefore in this experiment its value increased by 7 % during the first hour and thereafter remained constant.

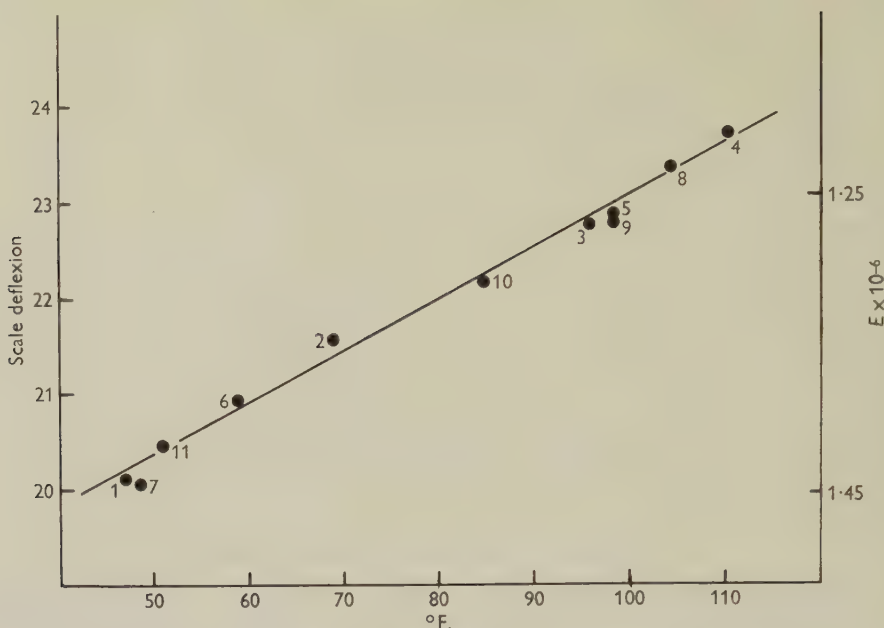
Evans & Lebow (1951) observed that in the human femur the value of Young's modulus for air-dried bone was 18 % greater than that for bone kept in tap water. This larger discrepancy may represent in part a species difference, but it seems probable that it is also related to storage in a hypotonic solution such as tap water rather than in physiological saline. On the other hand, Weir, Bell & Chambers (1949), examining rat femora, found a difference of under 2 % between the value of Young's modulus immediately after death and that after 2 weeks of air-drying. However, in the method these authors used, an appreciable interval must have elapsed after removal of the bone from the cadaver before any observations were made, and the measurement of Young's modulus was then made in air rather than in fluid.

The effect of temperature on the elasticity of bone

It is widely known that the elastic properties of many materials are dependent on temperature. The effect of thermal variations on the value of Young's modulus for bone was determined by the following method. A bone rod was arranged for cantilever loading and circulation of saline was established and maintained for 1 hr. to overcome the effects of evaporation during preparation. The circotherm pump was then set for a particular temperature; the temperature in the bath was allowed to reach a constant value and after an interval of 30 min., during which it was assumed that uniform heating of the bone would have occurred, the scale deflexion caused by the application of a known load for 2 min. was observed. The circotherm setting was then altered to another temperature and after another 30 min. interval the scale deflexion caused by the same load was again observed. In this way the scale deflexions caused by a standard load were observed at various temperatures in the range 40–110° F. It is evident from the equation on p. 505 that for a standard load the value of Young's modulus is inversely proportional to the scale deflexion. The results of the experiment can thus be expressed as in Text-fig. 8, in which Young's modulus in pounds per square inch and the scale deflexion in inches are plotted against the temperature in degrees Fahrenheit.

In this temperature range the relationship is approximately linear. The value of Young's modulus is inversely proportional to the temperature, decreasing from 1.45 at 40° F. to 1.25 at 99° F. The small figures alongside the points in Text-fig. 8 indicate the order in which the observations were made, and from this order it is evident that the effects of thermal variations on the elasticity of bone are fully reversible.

The same variations in the value of Young's modulus were noted when the experiment was repeated using the axial tensile stress method.



Text-fig. 8. The effect of temperature on the elasticity of bone.

The effect of the vascular pattern of bone on its elastic properties

During the present investigation the value of Young's modulus for bone from a number of different sources has been determined by two methods. The determination has been based on the one hand on the extension of a test piece caused by a pure tensile stress, and on the other, on the deformation by a bending stress of a test piece arranged as a cantilever. The values of Young's modulus assessed by these two methods will be denoted subsequently by E_T and E_B respectively.

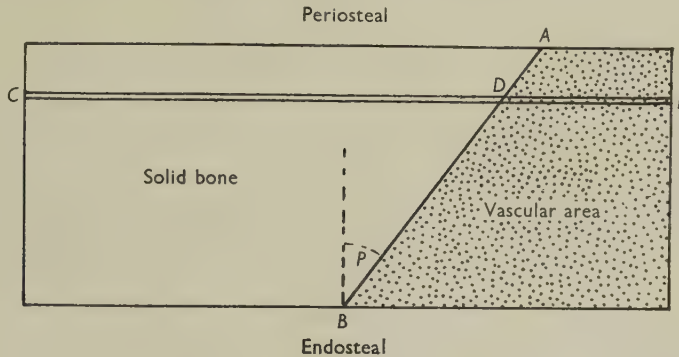
In homogeneous elastic materials such as steel the values of E_T and E_B are equal within the permissible limits of experimental error. Throughout the present study of bone, however, significant discrepancies between the two values have frequently been observed. When the discrepancy has existed E_B has always been less than E_T and the ratio E_T/E_B has varied in value between unity and a figure of about 1.7.

The usual equations expressing the reactions of materials to tensile and bending stresses are founded on the assumption that the material is homogeneous. It therefore seemed probable, at the outset, that the discrepancies between the values of E_T and E_B for bone might be related to one or more of the several heterogeneous features of the tissue. In bone the most obvious tissue-discontinuity is that due to the presence of vascular canals, and it is well known that the vascular pattern of that tissue varies considerably, not only in different species and at different ages but also in different regions in one bone.

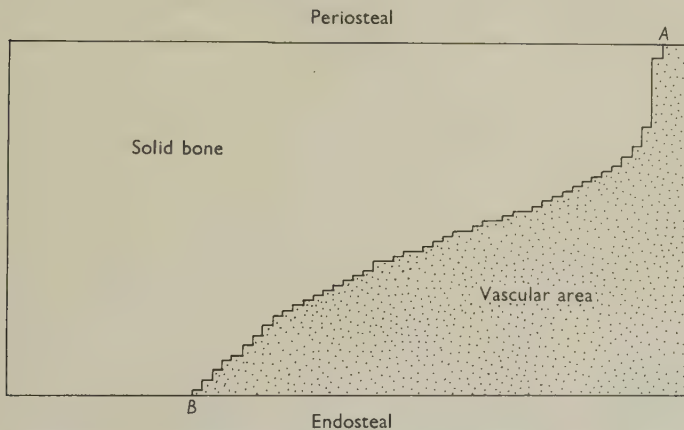
It has been observed that a high value of the E_T/E_B ratio is always associated with one of two well-defined vascular patterns. These two patterns will now be described and the mechanism of their effect on the values of E_T and E_B will be considered.

The high vascular gradient

Throughout some regions of bone the number and calibre of the vascular canals is fairly uniform. In other regions, however, there is an appreciable increase in the vascular field from the periosteal to the endosteal aspect. If the vascular area in a number of cross-sections through a test piece is summated along very narrow strips parallel to the periosteum, a diagram of a representative cross-section can be made (Text-fig. 9) in which the line AB indicates the proportion of vascular area to solid bone from the periosteal to the endosteal aspect.



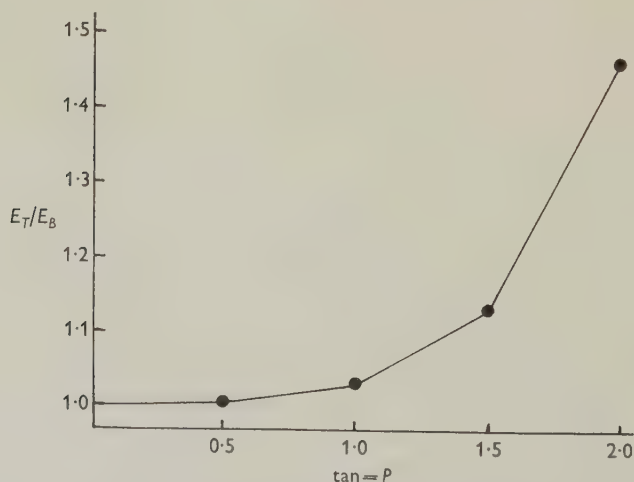
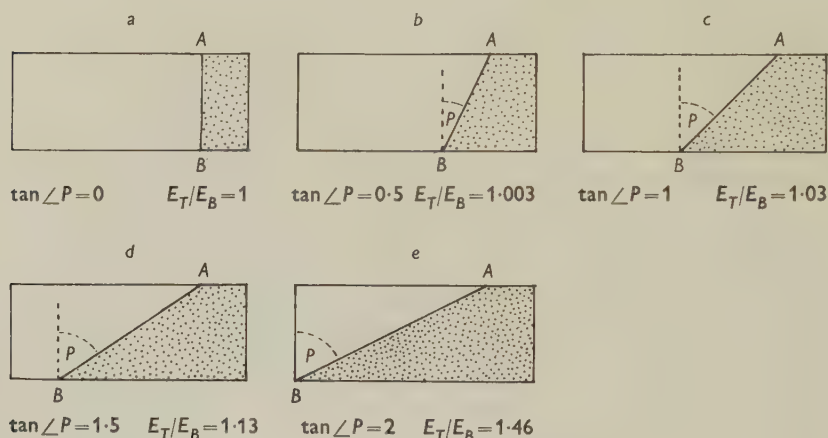
Text-fig. 9. Diagrammatic representation of the varying proportion of solid bone to vascular area in a bone rod.



Text-fig. 10. Variation in the proportion of solid bone to vascular tissue from the periosteal to the endosteal aspect of a bone rod from the tibia of a 72-year-old man.

When the line AB is perpendicular to both surfaces, the proportion of vascular area to solid bone is uniform throughout the test piece. Otherwise there is a vascular gradient between the periosteal and endosteal surfaces which may be expressed numerically as $\tan \angle P$. In practice of course AB is seldom a straight line and Text-fig. 10 shows the actual line in the case of a test piece from a 72-year-old human tibia. For the purpose of the present considerations, however, the diagram in Text-fig. 9 representing the most simple arrangement will be used.

It is evident that when a vascular gradient exists the value of Young's modulus must vary progressively from the periosteal to the endosteal aspect of the bone. In this analysis it is assumed that if E_S is the value of Young's modulus for solid bone, the modulus for the elementary lamina CDF in Text-fig. 9 is $E_S (CD/CF)$. Although this may be an over-simplification it does seem certain that the value of Young's modulus for the elementary lamina must be a function of CD and that the relationship suggested above is therefore adequate for a qualitative analysis. Text-fig. 11 shows a number of representative cross-sections, each 25 units by 10 units in area, in which the vascular gradient varies from zero in (a) to two in (e). Below each section is the ratio of the value of Young's modulus in tension (E_T) to that in bending (E_B) which is to be expected from purely theoretical considerations. In the graph in Text-fig. 11 the ratio E_T/E_B is plotted against the vascular gradient,



Text-fig. 11. Diagrammatic representation of five bone rods *a*, *b*, *c*, *d* and *e*. The vascular gradient and the E_T/E_B ratio calculated from it are indicated in each case.

and it is evident that in test pieces having a high vascular gradient of between one and two, the value of E_T will always be significantly greater than that of E_B . In other words, bone with a high vascular gradient is always more flexible than would be suspected from an examination of its extensibility.

The calculations involved in the determination of the ratio E_T/E_B in the case illustrated in Text-fig. 11*d* are outlined below as an example.

When such a test piece is subjected to a tensile load W , the intensity of tensile stress is W divided by the area of cross-section, i.e. $W/250$, and if ' e ' is the extension per unit length caused by that stress, then the value of Young's modulus in tension is

$$E_T = \frac{W}{250e}. \quad (2)$$

Consider the elementary lamina in the same test piece as shown in Text-fig. 12*a* during the same experiment. The lamina is parallel to and at a distance ' y ' from the endosteal surface and the area of its cross-section is $25dy$. If W_1 is the part of the total load W which the lamina supports, then the intensity of stress in the lamina is $W_1/25dy$. Furthermore if x represents the amount of solid bone in the lamina the value of its Young's modulus is $E_S X/25$, where E_S is the Young's modulus for solid bone. Now the extension of the lamina must be equal to that of the whole test piece and therefore

$$\frac{E_S X}{25} = \frac{W_1}{25dye},$$

or

$$W_1 = E_S ex dy.$$

However, the sum of the loads supported by all the elementary laminae making up the test piece is equal to the total load, and therefore

$$W = E_S e \int_0^{10} 10x dy.$$

Because of the inclination of the vascular gradient in this particular test piece

$$2x = 3y + 10,$$

therefore
$$W = \frac{E_S e}{2} \int_0^{10} (3y + 10) dy = \frac{E_S e}{2} [\frac{3}{2}y^2 + 10y]_0^{10} = 125E_S e.$$

But from equation (2), $W = 250 E_T e$.

Thus
$$\frac{E_T}{E_S} = \frac{125}{250} = \frac{1}{2}. \quad (3)$$

It is evident in Text-fig. 12*a* that the total area of solid bone is half the total area of the cross-section. Thus

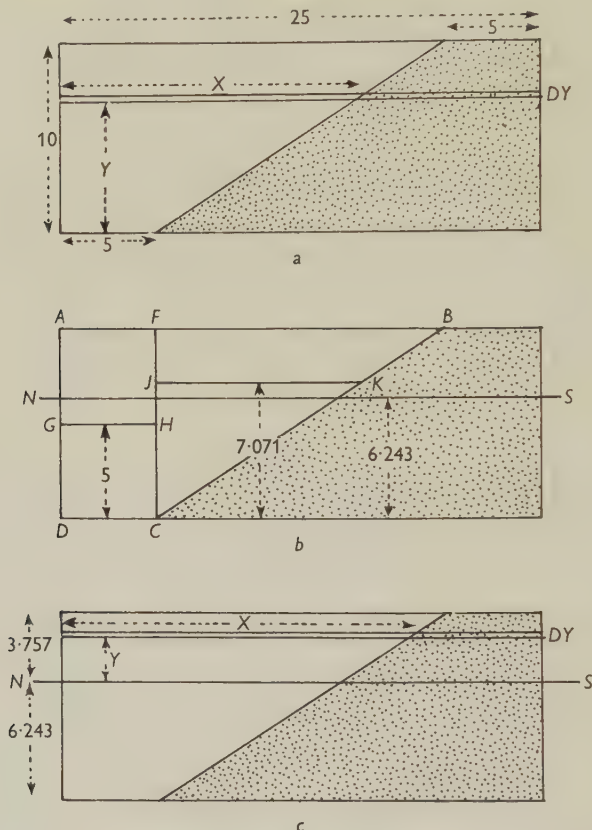
$$\frac{E_T}{E_S} = \frac{\text{area of solid bone}}{\text{area of cross-section}}.$$

When the same test piece is arranged as a cantilever, and bent in a plane perpendicular to the periosteal aspect, as described in a previous section (p. 504), the lower part is subjected to tensile stress and the upper part to compression stress. Somewhere between the periosteal and endosteal surfaces and parallel to them is a neutral surface or plane which is free of stress. This neutral surface traverses the centroid of each cross-section of the test piece and it is evident that the centroid of the whole cross-section must be in the same horizontal plane as the centroid of the area of solid bone $ABCD$ in Text-fig. 12*b*. The horizontal level of the centroid can be found by dividing the area $ABCD$ into rectangular and triangular parts by the line CF . It is apparent that the area of $AFCD$ is fifty square units

and that its centroid lies on GH , which is five units from DC . Similarly, the area of FBC is seventy-five square units and its centroid lies on JK , which is 7.071 units from DC . Thus the distance JH , the perpendicular distance between the centroids of $AFCD$ and FBC , is 2.071 units.

If NS represents the level of the centroid of $ABCD$ and therefore of the whole cross-section, and Z is the distance between NS and GH , then

$$50Z = 75(2.071 - Z) \quad \text{or} \quad Z = 1.243.$$



Text-fig. 12. Cross-sections of the bone rod illustrated in Text-fig. 11 (d).

Because the neutral surface of the bent test piece passes through the centroid, the representative cross-section can now be shown as in Text-fig. 12c with the neutral surface 6.243 units from the endosteal and 3.757 units from the periosteal surface.

Consider an elementary lamina in the compression zone of the test piece, lying parallel to and at a distance ' y ' from the neutral surface. Its area of cross-section is $25dy$ and its Young's modulus is $E_s X/25$. Because of the vascular gradient, in this zone

$$x = 1.5y + 14.365,$$

therefore

$$\frac{E_s x}{25} = \frac{E_s}{25} (1.5y + 14.365).$$

The intensity of stress in the lamina is

$$p = \frac{E}{R} y,$$

where R is the radius of curvature of the test pieces at the cross-section during bending, therefore

$$p = \frac{E_S}{25R} (1.5y^2 + 14.365y).$$

The moment of this stress about the neutral surface is the product of the intensity of the stress, the area of cross-section of the lamina, and its distance from the neutral surface or

$$\left[\frac{E_S}{25R} (1.5y^2 + 14.365y) \right] \left[25dy \right] \left[y \right] = \frac{E_S}{R} (1.5y^3 + 14.365y^2) dy.$$

The total amount of compression stress about the neutral surface is therefore

$$M_C = \frac{E_S}{R} \int_0^{3.757} (1.5y^3 + 14.365y^2) dy = \frac{328.5 E_S}{R}.$$

Similarly, the total moment of tensile stress about the neutral surface is

$$M_T = \frac{E_S}{R} \int_0^{6.243} (14.365y^2 - 1.5y^3) dy = \frac{595.3 E_S}{R}.$$

Thus the total moment of stress about the neutral surface is

$$M = M_T + M_C = \frac{923.8 E_S}{R}. \quad (4)$$

Now if E_B is the observed Young's modulus in bending, the bending moment is

$$M = \frac{E_B I}{R},$$

where I is the moment of inertia about a *central* plane and has a value of $\frac{1}{12} \times \text{width} \times \text{depth}^3$. Thus

$$M = \frac{E_B(25)(1000)}{12(R)} = \frac{2083.3 E_B}{R}. \quad (5)$$

It follows from equations (4) and (5) that

$$\frac{923.8 E_S}{R} = \frac{2083.3 E_B}{R}$$

$$\text{or} \quad \frac{E_B}{E_S} = \frac{923.8}{2083.3} = 0.4435. \quad (6)$$

Furthermore from equations (3) and (6) it follows that

$$\frac{E_T}{E_B} = \frac{0.5}{0.4435} = 1.13.$$

The same reasoning may be applied to actual test pieces, although because the vascular gradient is always irregular rather than linear, it is necessary to perform the summations by graphical methods. When this is done it is found that although the calculated E_T/E_B ratio approaches the observed value it does not usually equal it. Thus in one specimen the observed E_T/E_B ratio was 1.54, whereas the value calculated from the vascular gradient was 1.18, and in another specimen, whereas the observed value was 1.71 the calculated value was 1.3. It is evident therefore that although the presence of a high vascular gradient contributes towards inequality between E_T and E_B it is not the only factor involved.

Partial lamination of bone

In some specimens of bone the vascular pattern is such that it causes a partial lamination of the tissue so that laminae of practically solid bone alternate with relatively porous layers.

Thus in the early stages of its development bone is permeated by an extensive and labyrinthine vascular space. Although the form of this labyrinth is often quite irregular, in some regions it has a distinct preferential orientation, such that the intervening bone is in the form of thin sheets which lie parallel to the bone surface and have infrequent radial inter-connexions (Pl. 1, figs. 1, 2). Subsequently primary osteones form within this vascular labyrinth (Walmsley & Smith, 1959). The longitudinal vascular canals of these osteones lie within the sites occupied by the original vascular spaces and are consequently seen in transverse section to lie in rows parallel to the bone surface, areas of solid bone intervening between adjacent vascular rows (Pl. 1, fig. 3). In some regions, such as the posterior aspect of the horse radius, this stage of development persists into adult life. In other regions, however, the vascular canals of the primary osteones undergo local enlargement to form erosion cavities, and these are subsequently occupied by secondary osteones. Because the erosion phase of this reconstructive process usually proceeds excentrically from the canals of the primary osteones, the canals of the secondary osteones are usually not in alignment with the original vascular planes (Pl. 1, fig. 4). Consequently, as the reconstruction process becomes more extensive the subdivision of the bone into vascular and non-vascular laminae becomes progressively less and less distinct (Pl. 1, fig. 5) until in many adult mammalian bones it is no longer discernible.

It is evident that in regions of bone in which the primitive vascular spaces are circumferentially orientated (Pl. 1, figs. 1, 2) or in which the vascular canals of primary osteones have a circumferential alignment (Pl. 1, fig. 3), a longitudinal test piece will consist of layers of practically solid bone alternating with vascular laminae in which the proportion of bone is much less. In other words, there will be an alternation of strong and weak layers.

It has not been found possible to make any quantitative assessment of the physical results of such lamination, but the general effect which it has on the extensibility and flexibility of a tissue can be inferred from a consideration of the effects of complete lamination.

Thus if the homogeneous rod XYZ in Text-fig. 13 is subjected to a tensile load W , the intensity of tensile stress is W/YZ and the resulting extension could be denoted by e . The value of E_T would therefore be

$$E_T = \frac{W}{YZ} \frac{X}{e}.$$

If the rod is then divided longitudinally into say four equal laminae, each having the dimensions X , Y , $\frac{1}{4}Z$, and the four rods together are subjected to the same load W , the intensity of stress in each lamina is

$$\frac{W}{4(y\frac{1}{4}Z)} = \frac{W}{YZ}.$$

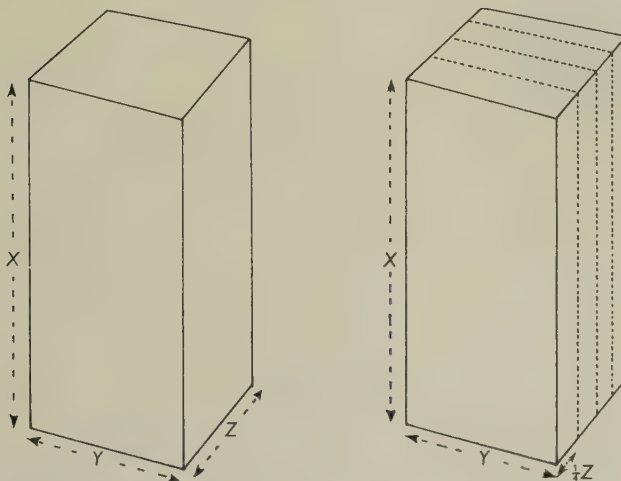
Thus the intensity of tensile stress is unaffected by lamination and therefore such subdivision will have no effect on the value of E_T .

On the other hand, if the solid rod is arranged as a cantilever as in Text-fig. 14a and subjected to a bending load W , the deflexion of the free end is

$$d_1 = \frac{4WX^3}{E_B YZ^3}.$$

When the laminated rod is bent in the plane of lamination the deflexion is

$$d_2 = \frac{4(\frac{1}{4}W)x^3}{E_B(\frac{1}{4}Y)Z^3} = \frac{4WX^3}{E_B YZ^3}.$$



Text-fig. 13. The effect of lamination of a rod on its extensibility.

But when laminated rod is bent at right angles to the plane of lamination as in Text-fig. 14b the deflexion is

$$d_3 = \frac{4(\frac{1}{4}W)x^3}{E_B Y(\frac{1}{4}Z)^3} = \frac{64WX^3}{E_B YZ^3}.$$

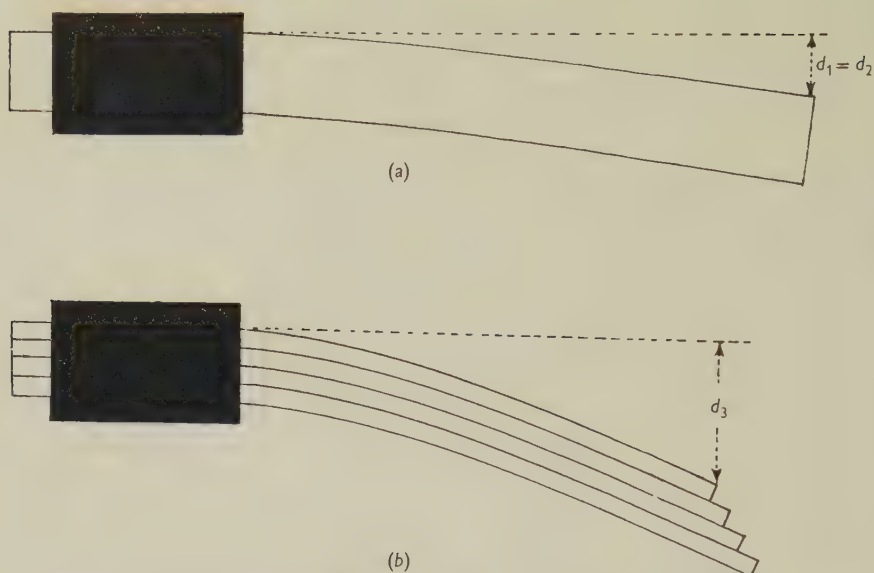
Thus longitudinal lamination of a tissue has no effect on its extensibility under tensile stress. In bending, the flexibility is unaffected when the bending occurs in the plane of lamination, whereas it is increased when the bending occurs at right angles to the plane of lamination.

These conclusions have been confirmed in practice. In a test piece taken from the adult human tibia in which the vascular pattern caused no lamination, the value of Young's modulus was determined in tension (E_T), in bending in a plane at right angles to the periosteum (E_{BA}) and in bending in a plane parallel to the periosteum (E_{BB}) and it was found that

$$E_T > E_{BB} = E_{BA}.$$

On the other hand, in a test piece from the posterior aspect of the adult horse radius in which the vascular pattern did cause partial lamination such as that illustrated in Pl. 1, fig. 3, it was found that

$$E_T > E_{BB} > E_{BA}.$$



Text-fig. 14. The effect of lamination of a rod on its flexibility.

DISCUSSION

It is apparent from the observations described above that if the value of Young's modulus is to have any significance as an index of the reaction of living bone to stress, certain criteria should be considered in its determination.

(1) The preparation of a bone rod for examination necessarily involves its removal from the normal fluid medium for about 10 min. During this time fluid is lost from the bone by evaporation and there is an associated change in the elastic properties. Fortunately both changes are reversed, probably completely, when the rod is returned to physiological saline.

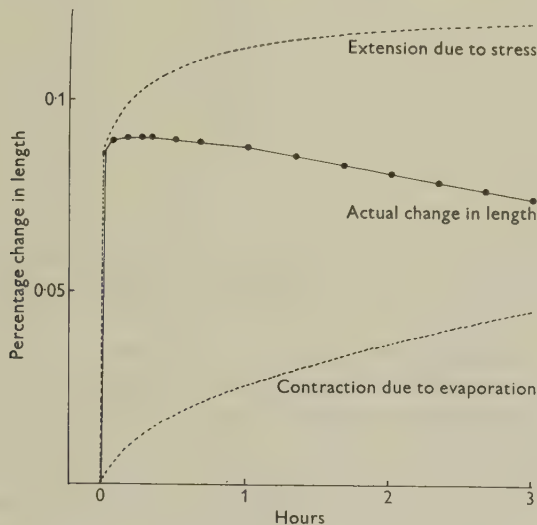
It is considered therefore that a bone rod should always be arranged for examination immediately after preparation and then maintained in physiological saline for an hour before any observations are made on its reaction to stress.

The error involved in determining Young's modulus in air is not in fact very great but if an attempt is made to observe the reaction of bone to *prolonged* tensile stress before evaporation is complete a very gross error is unavoidable. In these circumstances the progressive extension due to the *prolonged* load, and the progressive contraction which is associated with evaporation (p. 508) become algebraically summated and a result such as that shown in Text-fig. 15 may well be obtained.

(2) It is evident that if the calculated value of Young's modulus is to bear a close relationship to the response of bone to stress during life all observations must be made with the bone at the normal body temperature of the animal concerned.

It might be claimed that if all observations were made at room temperature there would be a standard error which would at least permit comparison of the moduli of different test pieces. But room temperature itself can vary appreciably and a variation of 30° F. would introduce an error of about 8%.

(3) In the presence of the phenomenon of elastic after-effect, the value of Young's modulus for bone is not a constant but a function of the stress duration. The duration of the stresses acting on bone during life varies within fairly narrow limits. At a minimum they are almost instantaneous, whereas in acts such as standing and sitting, which although apparently continuous are in fact essentially periodic (Smith, 1953), single stresses have an average duration of the order of one to two minutes. Ideally therefore Young's modulus for bone might be stated as a range rather than a single value, the limits of the range expressing the responses of the tissue to an instantaneous stress and to a stress of, say, 2 min. duration, i.e. $E^{0-2} = 2.0 - 1.8 \times 10^6$.



Text-fig. 15

In practice, however, it is virtually impossible to exert a known instantaneous stress and to measure the resulting deformation. It is suggested therefore that the deformation should be measured 2 min. after the application of stress. It is considered that this method has the merit that it fixes a standard duration of stress so that results of different experiments are comparable, and that the chosen duration is within physiological limits.

(4) It has been noted that the value of Young's modulus calculated by the cantilever method usually differs from that calculated from the extension caused by tensile stress. The latter is certainly the true value of Young's modulus for bone subjected to a longitudinal tensile stress. On the other hand, in life, long bones are not subjected to pure longitudinal tensile stress but almost invariably to bending stresses, and the behaviour of bone in these circumstances is more accurately reflected by the value of Young's modulus calculated by the cantilever method.

It seems more profitable to obtain an index which is of practical value rather than one which is academically correct and it is therefore suggested that Young's modulus for bone should be calculated from the deflexion or angulation of a rod arranged and loaded as a cantilever.

When these criteria are accepted it is found that the value of Young's modulus for bone is considerably lower than the values determined by previous workers. Thus in seventeen test pieces taken from the medial surface of the human tibia at post-mortem, the value of Young's modulus varied between 1.15 and 2.02×10^6 lb./sq.in. with an average value of 1.54×10^6 .

This result is compared in Table 1 with the value of Young's modulus for the human femur and tibia, obtained by other authors.

Table 1

Author	Bone	Condition	Type of stress	$E \times 10^{-6}$
Wertheim (1847)	Femur	Dry	Tension	3.22
Raubert (1876)	Femur	Dry	Tension	2.90
	Tibia	Dry	Tension	2.67
Carothers <i>et al.</i> (1949)	Femur	Preserved	Compression	2.67
	Tibia	Preserved	Compression	2.84
Evans & Lebow (1951)	Femur	Dry	Tension	2.61
	Femur	Wet	Tension	2.08
Present study	Tibia	Wet	Bending	1.54

SUMMARY

The factors affecting the elasticity of bone in bending and in tension have been investigated and a procedure is suggested for the determination of the value of Young's modulus for this tissue.

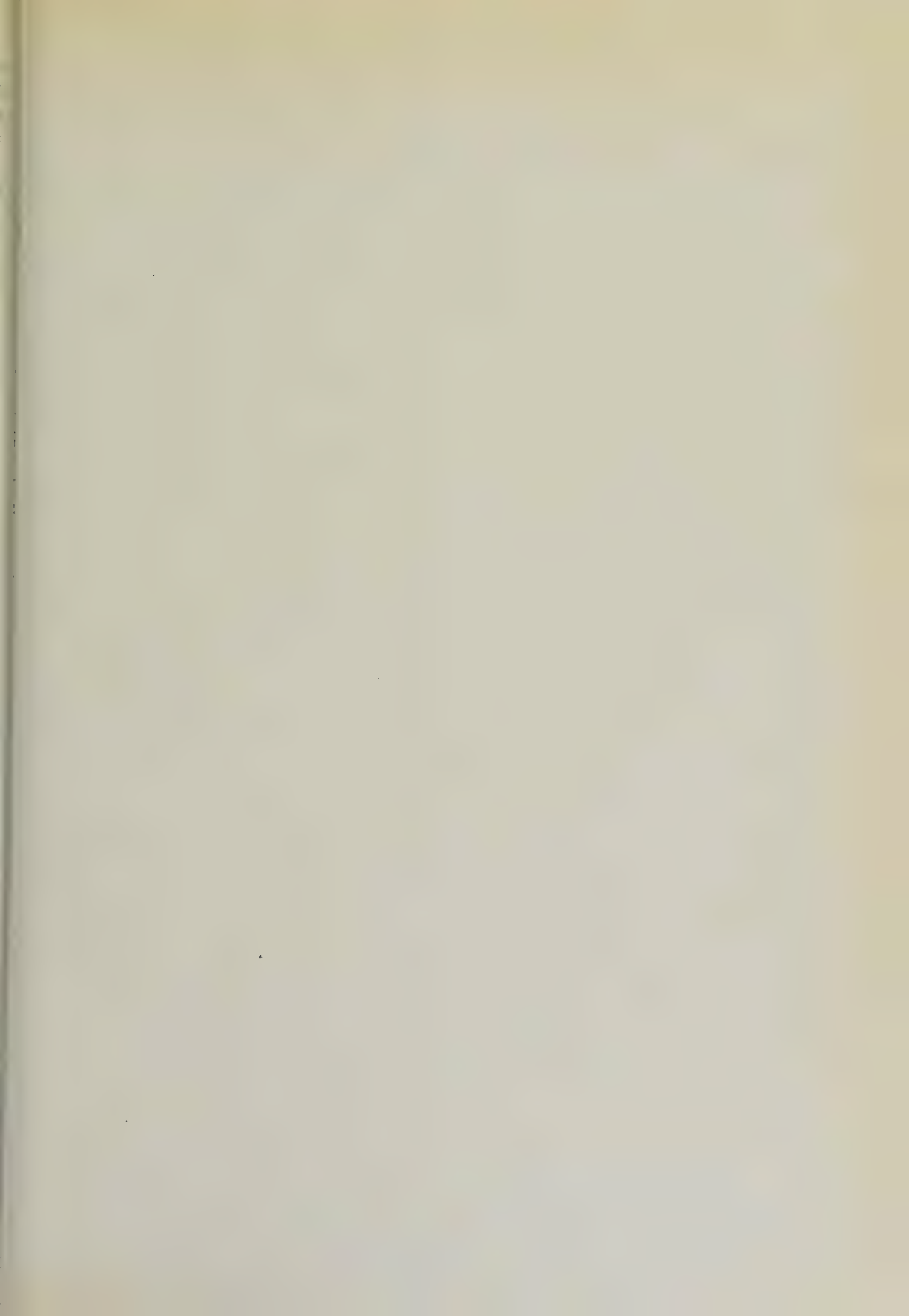
The deformation of bone under stress varies with the stress duration, with the fluid content of the specimen and with its temperature.

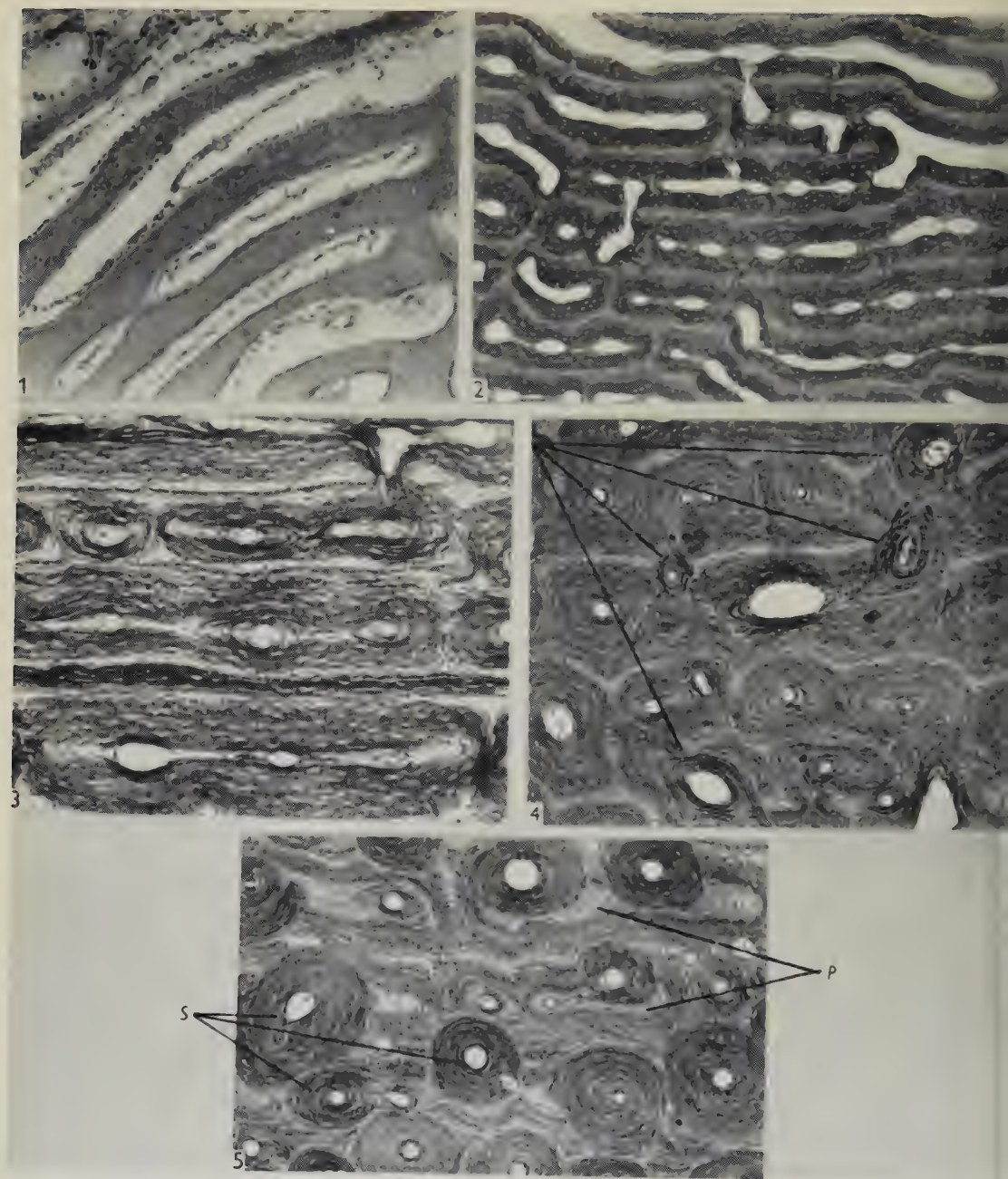
The relationship of the modulus in tension to the modulus in bending is dependent in part at least on the vascular pattern of the bone.

We wish to express our thanks to Prof. Dick of the Department of Civil and Mechanical Engineering, Queen's College, Dundee, for his advice, and to Mr J. Brown who prepared the photographs in the plate. We wish to acknowledge a grant made to us by the Carnegie Trust for the Universities of Scotland towards the cost of the reproduction of the plate.

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EXPLANATION OF PLATE

- Fig. 1. T.S. Femur of 7-month human foetus. Haematoxylin and eosin, $\times 140$.
- Fig. 2. T.S. Metacarpus of 2-month-old ox. Weidenreich's modification of Weigert's fibrin stain, $\times 70$.
- Fig. 3. T.S. Posterior part of adult horse radius. Weidenreich, $\times 65$.
- Fig. 4. T.S. Posterior part of adult horse radius. The pointers indicate secondary osteones forming without the horizontal vascular planes of the primary osteones. Weidenreich, $\times 65$.
- Fig. 5. T.S. Anterior part of adult horse radius. The partial lamination of the bone associated with the primary osteones (*P*) has been largely destroyed by the formation of numerous secondary osteones (*S*). $\times 65$.

POSTURAL MOVEMENTS DURING NORMAL STANDING IN MAN

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INTRODUCTION

The characteristically erect standing posture of man has attracted systematic study, from a variety of points of view, for well over half a century. The original contention of Braune & Fischer (1889), that the transverse joint axes and the centres of gravity of the body parts all lie in a single coronal plane including the lower ankle pivots of the whole body, was not substantiated by subsequent investigations. It is now generally recognized that, during normal standing, the centre of gravity of the whole body mass plumbs to a point on the ground which is on the average a few cm. in front of the transverse ankle axis (Hellebrandt & Franseen, 1943; Morton, 1952). There appears to be no recent discussion of the disposition of the centres of gravity of the individual body parts during normal standing.

Normal standing is not, however, a static posture. That the body is in continuous motion, even when the attempt is made to stand quite still, was first established experimentally by Vierordt (1862). Subsequent investigations of this postural sway, predominantly as a test of psychomotor function, have assessed the degree of the sway chiefly by recording movements of the head in the horizontal plane (Miles, 1922, 1950). Hellebrandt (1938) recorded movements of the centre of foot pressure on the ground, with the implication that these movements were directly related to movements of the vertical projection of the centre of gravity of the whole body. Smith (1957) recorded antero-posterior movements at approximately hip level.

Possibly on account of the technical difficulties involved, there does not appear to have been a detailed investigation of the simultaneous movements of the different parts of the body during postural sway, and it has sometimes been assumed that the body remains effectively rigid above the ankles, joint movement being virtually restricted to the ankle joints (Smith, 1957). Expression of variation between subjects in the extent of postural sway has commonly been restricted to specification of the distance between the extreme limits of sway during a specified standing period, of the horizontal area of the limits for antero-posterior and lateral sway combined, or of the total distance traversed by a fixed point on the body (usually on the head) in the horizontal plane. Such specifications are not generally amenable to further mechanical analysis, and the measure of postural sway provided is frequently empirically dependent on the details of construction of the recording apparatus. A definition of postural sway in mechanically analysable terms is obviously necessary before an attempt can be made to infer details of the muscular mechanism underlying postural maintenance, or as a preliminary to the investigation of the effects

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on the postural mechanism of practical situations, such as the carriage of loads in the hands or on the back.

A feature of the postural mechanism during normal standing which has received some attention in the past is the dependence of the standing posture on muscular activity. Even before the experimental demonstration of postural sway it was contended by Weber & Weber (1836) that the erect posture was maintained in man not by muscular activity, but by non-active ligamentous tension. Recent investigators (Clemmesen, 1951; Ralston & Libet, 1953) have sometimes tended to revert to this conception of postural maintenance against gravitation by 'passive elastic tension'. This recent view has been based on electromyographic studies which appear to demonstrate the electrical 'silence' of postural muscles during standing. Other electromyographers, however, report continuous, or almost continuous electrical activity during standing, particularly in the extensor, antigravity musculature (Jacobson, 1943; Joseph & Nightingale, 1952; Joseph, Nightingale & Williams, 1955; Portnoy & Morin, 1956). A definition of the events of postural sway in mechanical terms should obviously assist in deciding whether or not these events are possible in the absence of muscular driving forces. Smith (1957), assuming that motion occurs only in the ankle joints during standing, has calculated some of the muscular forces which would be required to maintain the motion observed.

The present investigation was designed as an attempt to describe some of the events of postural sway in a more objective and mechanical manner and, thereby, to re-assess the previous assumptions of body rigidity and absence of muscle action during normal standing.

METHODS

A force analysis platform (Whitney, 1958*a*) was used to obtain, for a subject standing normally on the platform, a continuous record of the horizontal forces of reaction at the feet and the co-ordinates, on the horizontal surface of the platform, of the centre of foot pressure (hereafter referred to as the CFP). The construction of the platform has been described elsewhere (Whitney, 1958*a*). The surface, 1 m. square, on which the subject stands, is elastically suspended about 25 cm. above the ground and its horizontal movement is elastically restrained. Identical suspension and restraining systems are provided at each of the four corners of the platform, so that a force applied in any direction at any point on to the surface is distributed, in accordance with the usual rules of statics, to the four corners to produce proportionate deflexions in the suspending and restraining systems. The mechanical arrangements are such that the deflexions are resolved along three rectilinear axes, the axes being usually related to the anatomical axes of the subject standing on the platform and they will be referred to subsequently as vertical, antero-posterior and transverse. The deflexions along the three axes at each corner of the platform are electrically transduced by means of resistance strain gauges incorporated in the suspension, so that the force exerted along a particular axis at a particular corner produces a proportionate change of electrical resistance in the gauge provided in the axis. Gauges for corresponding axes at the four corners of the platform are combined into Wheatstone bridge circuits, so that the electrical output of an energized bridge circuit is made proportional to the desired parameter of the force applied to the

surface of the platform. Three simple addition circuits provide outputs which are proportional to the vertical, antero-posterior and transverse components of the reactionary force at the feet of a subject standing on the platform. The co-ordinates of the CFP in the horizontal surface of the platform, relative to a known origin (actually

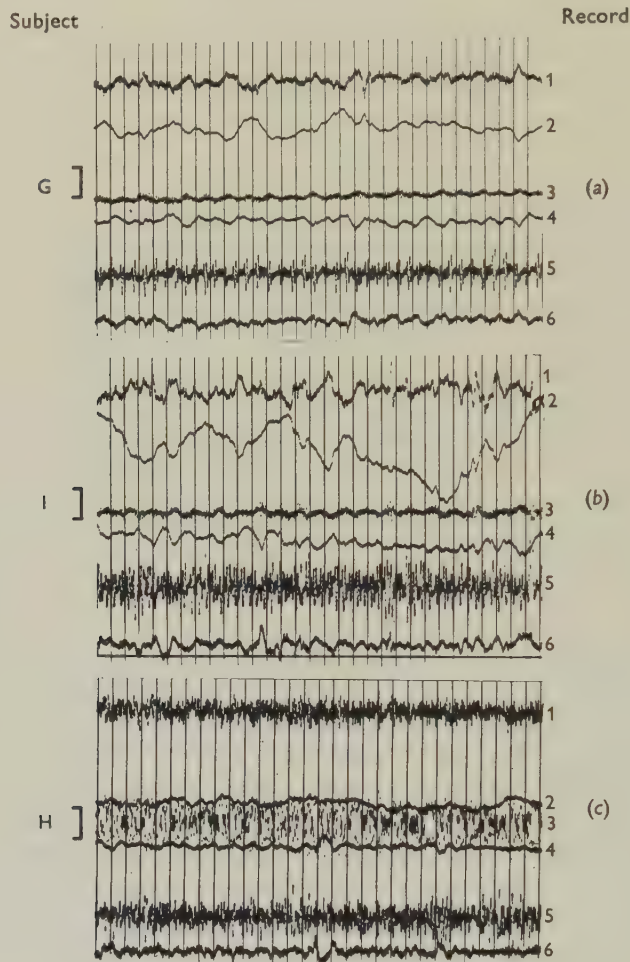


Fig. 1. Records of postural movements and forces at the feet obtained with the Force Analysis Platform. Samples of records obtained for three subjects (*G*, *I* and *H*, from above downwards), illustrating the variation in the patterns of postural activity. Trace identification for each record (reading from above downwards), and the approximate calibration values for an upward deflexion equal to the scale interval on the left: (1) Antero-posterior force of reaction at the feet (F), calibration 400 g. wt. directed posteriorly. (2) Antero-posterior co-ordinate of centre of foot pressure (Y), calibration 1 cm. for an 85 kg. body weight, directed anteriorly. (3) Acceleration of Platform (output of Platform-Mounted Accelerometer). Calibration 2 cm./sec.², directed anteriorly. (4) Transverse co-ordinate of centre of foot pressure, calibration 400 g. wt. directed downwards. The record is predominantly the ballistocardiogram during standing. (6) Transverse force of reaction at the feet. Calibration as for Trace 1, directed to left side. The vertical timemarks in all records were at 1 sec. intervals.

the centre of the platform surface), are provided by suitable additions and subtractions carried out by two further bridge circuits incorporating separate sets of gauges for the vertical axes. The mechanical electrical arrangements ensure that the transducing systems for the five platform parameters are appropriately independent. The bridge circuits are A.C. energized for carrier amplification of the outputs, which are then recorded by a photographic, multichannel, galvanometer recorder (Films and Equipments Ltd.). Each parameter is directly calibrated by static loading of the platform. The type of record obtained for a subject standing normally on the platform is illustrated in Fig. 1. These records include a sixth platform parameter obtained as the output from an accelerometer (Statham Model C-1-350) which was rigidly attached to the suspended platform with its sensitive axis parallel with the antero-posterior axis. With this mounting the output of the accelerometer is proportional to the acceleration imparted to the platform along the antero-posterior axis by the antero-posterior component of the reactionary force at the feet (F). Thus, the accelerometer output is proportional to the second derivative (double differentiation) of the force F , which, as will be apparent from Fig. 1, has high frequency components of small amplitude ('tremor') superimposed on low frequency components of much greater amplitude. Double differentiation of this mixed waveform has the mathematically expected result of selectively attenuating the low frequency components, so that the tremor could be amplified and recorded at analysable magnitude without incurring the risk of deflexion of the record beyond the limits of recording paper width.

Although the suspension and restraint of the platform is elastic, the system is actually very 'stiff' (deflexion along any of the three axes 0.0017 cm./kg. wt.). The deflexions are not perceptible to the subject, even if he performs active movements whilst standing on the platform. For a subject standing still the platform is indistinguishable from a quite firm footing. In addition, deflexions of the platform are optimally damped by hydraulic means to eliminate self-oscillation and feedback of the deflexions to the subject.

Records similar to those illustrated in Fig. 1, obtained for a number of subjects during normal standing, showed that postural activity along the antero-posterior axis was generally much more prominent than that along the transverse axis. Also, along the vertical axis, postural forces were confused with ballistocardiographic forces. Records for detailed analysis therefore included only three of the platform parameters—the antero-posterior reactionary force at the feet (F), the output of the platform-mounted accelerometer, and the antero-posterior co-ordinate of the CFP (Y). Fig. 3*a* shows a representative record.

Movement of the trunk in the sagittal plane was recorded by attaching the accelerometer to a belt mounted on the subject at umbilical level (Fig. 2). With the sensitive axis of the accelerometer oriented approximately horizontally in the sagittal plane, the output for a subject standing normally (Fig. 3*b*) shows large deflexions of low frequency with high frequency tremor superimposed. It follows, from the principle of the accelerometer, that the large deflexions in the record indicated changes of inclination of the accelerometer axis with respect to the vertical direction of gravitation, and these deflexions could therefore be calibrated to derive changes in trunk inclination at the umbilical level. The superimposed deflexions at

high frequency indicated the instantaneous acceleration of the trunk, in an antero-posterior direction and at umbilical level.

The subjects for the experiments were 10 male students, ages 19–20 years, free from obvious physical disability and from any recent history of skeletal or muscular injury.



Fig. 2. Posterior view of accelerometer mounted on belt at umbilical height, for recording the antero-posterior acceleration of the trunk and variations in trunk inclination.

During the standing experiments the subjects wore vest, shorts and rubber plimsolls (heel thickness about 1.5 cm.). The data given in Table 1 include this clothing. In this, and in all subsequent tables, the subjects are listed in order of increasing stature so that variations in the parameters of postural activity may be readily related to stature if required.

The heights of the centres of gravity for the subjects, quoted in Table 1, were experimentally determined by mounting a stretcher horizontally on the force analysis platform, the axis of the stretcher being aligned with the Y-co-ordinate axis of the platform. The position of the centre of gravity of the stretcher, with and without a subject lying on it, was then determined in relation to the calibrated

Y-axis. The stretcher was provided with a vertical foot-board, against which the subject pushed with the fully extended lower limbs whilst the necessary readings were taken. The dimensional relationship between the Y-axis and surface of the foot-board being known, the height of the centre of gravity above the soles of the feet could be determined.

Each subject was examined on two occasions (Serials 1 and 2) which were separated by a period of up to two weeks. On each occasion records were taken during a standing period of 4 minutes. The subject stood at ease on the platform, with

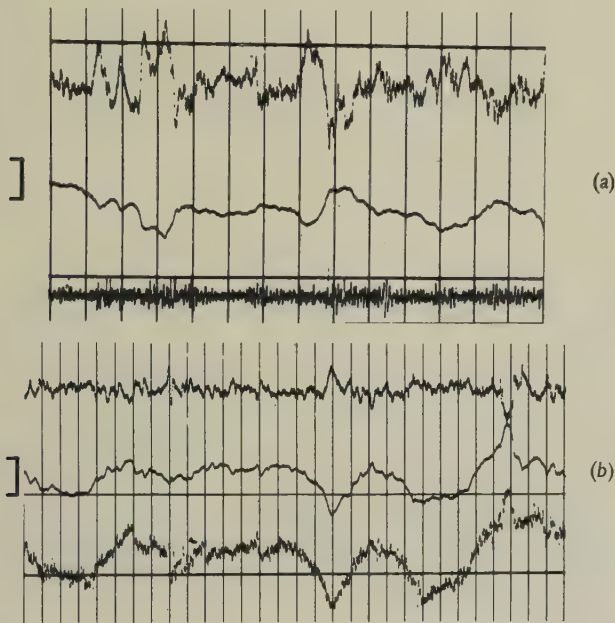


Fig. 3. Examples of the two types of record used for the quantitative analysis of postural movements. Trace identification of upper record, *F*, *Y*, and platform acceleration from above downwards. Calibration of the records as for Fig. 1, but *F* channel was operated at twice the sensitivity. Trace identification of lower record, *F*, *Y* and trunk-mounted accelerometer, from above downwards. Calibration of *F* and *Y* as for Fig. 1. Calibration for accelerometer: 0.012 radians forward inclination or 11 cm./sec.² rearward acceleration of the trunk. The fitted baselines are shown in the lower record, for *Y* and trunk inclination. These baselines apply for a 2.5 min. run of record, and they do not necessarily fit the portion of record reproduced. The positions of the two baselines in (a), which were produced by optical markers incorporated in the recorder, are not significant. Time marks are at 1 sec. intervals, (a) being recorded at twice the paper speed of (b).

the arms loosely hanging, the heel centres 20 cm. apart, and the toes spread at the preferred angle. The subject was instructed to stand still, looking straight ahead at a non-uniform visual field with no object in the field nearer than 4 m. The posture was not otherwise controlled, and the subject was not informed of the real object of the experiment—he was actually told that his ballistocardiogram was being recorded. During the first minute of each standing period the accelerometer was mounted on

the platform and was transferred to the belt on the subject without appreciable disturbance, for the subsequent 3 min. Fig. 2 gives examples of the two types of record obtained.

Table 1. *Data on subjects*

Subject	Age	Stature (cm.)	Body weight (kg.)	Height of centre of gravity from ground	
				(cm.)	(% stature)
A	20	172.7	65.4	97.7	56.5
C	19	174.9	79.1	95.9	54.9
E	19	176.2	74.0	99.2	56.2
G	19	177.2	66.4	99.4	56.1
D	20	178.6	74.3	99.9	56.0
H	19	179.1	69.7	99.3	55.5
K	19	184.3	63.2	103.0	56.0
B	20	186.4	83.5	101.7	54.5
I	19	188.4	78.5	105.0	55.7
J	20	192.1	83.5	105.4	54.9
Means	19.4	181.0	73.8	100.7	55.6

ANALYSIS AND RESULTS

The general object of the analysis was to specify, in quantitative terms, the antero-posterior movements which occur in the body during normal standing. cursory examination of the records revealed that movement, whether of trunk inclination or displacement of centre of foot pressure along the antero-posterior axis, is cyclical in nature. The low frequency components of this cyclical movement are generally of larger amplitude and greater irregularity than the high frequency components. The high and low frequency components appear to be quite distinct on the records of trunk inclination, and they were independently analysed from the same record. Only the low frequency movements were represented on the CFP records in analysable magnitude since amplification adequate to reveal the high frequency components was precluded by the large amplitude at low frequency. The examination of the high frequency part of the postural activity referred to the feet therefore depended on analysis of records obtained from the accelerometer mounted on the platform.

The method of analysis used for the low frequency deflexions on the records of trunk inclination and CFP (Fig. 3*b*) was as follows. A baseline was fitted to a 2.5 min. run of each record so that the total areas included between baseline and curve were equal above and below the baseline. The total area above or below the baseline for the 2.5 min. run was also determined. These operations were carried out in the usual manner with an 'Allbrit' Ruleform Planimeter. The mean deflexion of the curve above or below the fitted baseline was obtained by dividing the total area by the measured length of the 2.5 min. run of record. The mean deflexions, expressed as cm. on the record, were then converted to angular measure (in the case of trunk inclination) and to cm. of actual antero-posterior movement (in the case of CFP) by reference to calibration factors. The mean deflexions are summarized in Tables 2 and 3. In the case of the CFP record the extent of the maximum movement about the baseline during each 2.5 min. run was measured and, multiplied by the appropriate calibration factor, is given in Table 2 as the 'Maximum Sweep'. The frequency of the movements of the CFP about the fitted baseline was also obtained

by direct counting and is given in Table 2. The frequency of movement indicated by the trunk inclinometer was found to be substantially the same as that derived from the corresponding CFP record.

Table 2. *Antero-posterior movement of centre of foot pressure*

Subject	Average frequency (c.p.s.)		Average amplitude (cm.)		Maximum sweep (cm.)		Sweep/amplitude	
	1	2	1	2	1	2	1	2
A	0.12	0.15	0.44	0.38	1.91	1.62	4.3	4.3
C	0.28	0.29	0.36	0.42	1.88	1.91	5.3	4.5
E	0.22	0.24	0.48	0.39	2.33	1.74	4.8	4.4
G	0.12	0.13	0.96	0.58	3.01	2.20	3.2	3.8
D	0.39	0.21	0.48	1.13	2.59	4.31	5.4	3.8
H	0.14	0.26	0.46	0.60	1.84	2.68	4.0	4.5
K	0.19	0.24	0.72	0.61	3.62	3.40	5.0	5.6
B	0.20	0.30	0.58	0.45	2.75	2.37	4.7	5.3
I	0.15	0.16	0.87	1.00	3.80	4.83	4.4	4.9
J	0.24	0.16	0.66	0.62	3.30	3.45	5.0	5.6
Means	0.21	0.21	0.60	0.62	2.70	2.85	4.5	4.6

Notes on Table 2

1. The values quoted are based on the analysis of 2.5 min. of normal standing.
2. The column sub-headings '1' and '2' refer, respectively, to the first and second occasions of standing.
3. The subjects are listed in order of increasing stature.
4. The values quoted for the average amplitude were obtained from the corresponding values for the planimetric mean deflexion by using a constant multiplying factor $\pi=3.14$ (see text for details).

Table 3. *Variation of antero-posterior trunk inclination and relative movement about hip and ankle joints*

Subject	Average amplitude (0.01 radian)		Ratio: expected/actual inclination (τ)		Ratio: hip/ankle rotation (e)	
	1	2	1	2	1	2
A	0.80	0.59	1.77	1.53	1.29	0.80
C	0.79	0.68	2.13	1.55	1.90	0.75
E	0.90	0.54	1.84	1.37	1.35	0.50
G	1.33	1.14	1.39	1.96	0.58	1.76
D	1.14	0.99	2.36	0.88	2.20	0.12
H	0.82	0.95	1.77	1.57	1.29	0.80
K	1.25	1.13	1.79	1.89	1.27	1.41
B	0.64	0.63	1.13	1.43	0.18	0.73
I	—	1.47	—	1.77	—	1.27
J	—	1.01	—	1.63	—	0.91
Means	0.96	0.89	1.77	1.56	1.25	0.88

Notes on Table 3

1. See Notes 1-4 for Table 2.
2. For details of the derivation of ratios τ and e , see text.

The mean amplitude of the tremor recorded from the accelerometer mounted on the trunk or on the platform was also obtained by planimetry, the area of the tremor envelope for a known length of record being measured and the mean height of the envelope deduced. One half of the mean envelope height, expressed in acceleration units, then gave the mean amplitude. The results are summarized in Table 4. The frequencies of tremor, obtained by counting the number of complete cycles over

a known time interval of the record, are also summarized in Table 4. In all cases, both for low and high frequencies, frequency is given as number of complete cycles per second (c.p.s.).

The large, low frequency, antero-posterior movements. As will be apparent from Fig. 3, these movements were characteristically irregular, both as regards amplitude of movement and duration of cycle. Despite the approximate and empirical method adopted, in deriving the frequencies of the large movements, it is apparent from Table 2 that the results obtained on the two occasions were reasonably consistent for the different subjects, indicating that the frequency is to some extent characteristic of the subject. The range of frequencies (0.12–0.39 c.p.s.) for all subjects and occasions was quite large. The general value for the frequency of movement appears to be about 0.2 c.p.s. (that is, one complete cycle of sway in 5 sec.), and a similar frequency was suggested by Smith (1957). However, the extreme irregularity of the low frequency movements must again be emphasized, so that it is difficult to infer any functional significance for this type of movement. It seems reasonable to conclude, however, that such irregularity would not arise if the movements were of purely mechanical origin, and pendular in nature.

Table 4. *The high frequency components of the antero-posterior trunk movement and of the reactionary foot force*

Subject	Trunk movement			Force at feet		
	Frequency (c.p.s.)	Amplitude trunk accn. (cm./sec. ²)	Amplitude trunk movement (0.001 cm.)	Frequency (c.p.s.)	Amplitude platform accn. (cm./sec. ²)	Amplitude force at feet (g. wt.)
A	10.3	2.12	5.04	15.9	0.23	12.9
C	12.4	1.85	3.03	14.4	0.13	8.7
E	11.3	1.78	3.54	15.7	0.15	8.9
G	12.1	2.39	4.14	17.7	0.17	6.2
D	9.9	4.45	12.08	16.8	0.47	23.9
H	9.8	3.86	10.39	11.3	1.16	138.9
K	12.5	4.39	6.95	18.7	0.17	6.9
B	10.0	2.27	5.77	16.9	0.14	6.7
I	13.2	2.10	3.62	17.0	0.24	11.8
J	12.2	3.04	5.46	13.4	0.48	38.7
Means	11.4	2.83	6.16	15.4	0.34	26.4

Notes on Table 4

1. The subjects are listed in order of increasing stature.
2. Each value quoted is the mean for the two occasions on which each subject was examined.

The definition of the extent or amplitude of the low frequency postural movements is complicated by the irregularity of the record of displacement against time. The lack of apparent repetition of amplitude and duration from cycle to cycle suggests that these parameters are randomly distributed about mean values, and the derivation of the mean value for the frequency has already been discussed. The derivation of the mean amplitude of the movement from the planimetric mean deflexion, obtained in the manner described from the records of trunk inclination and CFP, depends on the shape that can be assumed for the waveforms indicated on the records. If the waveforms are assumed to be sinusoidal, then the arithmetic mean amplitude would be given by: ($\pi \times$ planimetric mean deflexion). Although

the waveforms are clearly not perfectly sinusoidal, the factor by which the planimetric mean deflexion should be multiplied to give the mean amplitude should not be greatly different from π ($=3.14$) for small departures from the sinusoidal—the factor would become 4.00 for a perfect ‘saw-tooth’ waveform and 2.56 for a perfectly semi-circular one. The actual waveforms for the postural movements clearly lie within these limits, so that the adoption of π as the appropriate factor seems reasonable and has been used to derive the mean amplitudes quoted in Tables 2 and 3. This method of determining the mean amplitude of postural movements, and the conception that the amplitude varies in a random manner from cycle to cycle, receives some support from a comparison of mean movement during a standing period with the maximum movement during that period. If the amplitude measured from the baseline varied randomly from zero to some maximum, then it would be expected that the maximum sweep about the baseline would be four times the mean amplitude on either side of the baseline. Table 2, for the movements of the CFP, shows that this expectation was closely realised, the maximum sweep being, on the average, about 4.5 times the mean amplitude.

Tables 2 and 3 show that the mean amplitude of the centre of foot pressure (CFP) movement antero-posteriorly varied from 0.36 to 1.13 cm. for the different subjects and occasions; and that the mean amplitude of the trunk inclination varied from 0.54 to 1.33 (0.01 radian units, 1×0.01 radian unit being equivalent to 0.57° of arc). Fig. 3*b* also shows that there was, as might have been expected, a general correspondence between movements of the CFP and changes of trunk inclination. Movements of the CFP must reflect corresponding antero-posterior movements of the centre of gravity (CG) of the body, the CG being located at a height (H cm.) above the platform. The experimental value of H for the different subjects is given in Table 1. If the body swayed as a rigid rod about the ankle pivots then:

$$\frac{(\text{Mean amplitude trunk inclination in radians}) \times H}{(\text{Mean amplitude CFP in cm.})} = r = 1.$$

It will be seen from Table 3 that the ratio ‘ r ’ was nearly always greater than unity, the actual range of values for ‘ r ’ being 0.88 to 2.36. The obvious explanation for this result is that, during sway, rotation of the trunk relative to the lower limbs occurs in addition to, and generally in the same direction as, rotation of the whole body about the ankle pivots. If it is assumed, as seems reasonable, that the trunk rotates relative to the legs about an axis roughly coincident with the hip joints, it is possible to estimate, for a given record, the relative amounts of rotation about the hip and ankle pivots. In making this estimate it is necessary to take into account the distribution of body mass above and below the hip joint, and also the location of the hip joint relative to the ground. For these values use has been made of the data provided by Dempster (1956), based on the dismemberment of cadavers. From this source it appears that 70 % of the body mass is carried above the hip joint and has its centre of gravity a distance equal to 20 % of the stature above the hip joint. The centre of gravity of the legs (30 % of the body mass) is located a distance equal to 28 % of the stature above the ground. The centre of gravity of the whole body is 56 % of the stature above the ground—this figure was confirmed for the subjects under discussion (see Table 1). Also the hip joint is 50 % of the stature above

the ground. These values are, of course, approximate, for some variation must occur in different body builds, but their use leads to a comparatively simple formula for the average ratio of hip to ankle rotation during standing:

$$\frac{\text{Hip rotation}}{\text{Ankle rotation}} = e = \frac{(4r-4)}{(4-r)}$$

where r is derived from the mean amplitudes of trunk inclination and CFP movement in the manner already described. The derived values of e are given in Table 3. It will be apparent that there is some variation in the relative movement at hip and ankle but, in general, the angular movement is about equal ($e = 1$). The standing body does not sway about the ankles as a rigid rod, but 'as a reed in the breeze'.

In the above discussion of the low frequency components of antero-posterior body sway it has been assumed that the movements of the CFP accurately reflect the antero-posterior movements of the CG of the body mass. This assumption has generally been made by previous workers (e.g. Hellebrandt, 1938). The coincidence of the CG and CFP in the same coronal plane is valid only for the static situation. For the actual dynamic situation of cyclical swaying the amplitude of the CFP movements will be greater than that of the CG movements, and the difference will increase with increase of the frequency of the swaying movement. If the swaying were sinusoidal, of constant amplitude and constant frequency (n c.p.s.), the following relationship would be obtained from simple dynamical considerations:

$$\frac{\text{Amplitude of CFP}}{\text{Amplitude of CG}} = (1 + 4\pi^2 n^2 k_0^2 / gH)$$

where k_0 is the radius of gyration of the body about the ankle pivot, H is the height of the CG of the body and g is the acceleration due to gravity (981 cm./sec.²). The employment of the mean frequencies (Table 2) of the irregular motion of body sway indicates that the movements of the CFP may amplify the actual movements of the CG of the body by as much as 60 % for a frequency of swaying of 0.4 c.p.s. On the average exaggeration indicated by substitution of the mean frequency in the above formula was about 20 %. Somewhat similar considerations apply for the record of trunk inclination, but the exaggeration in this case was less (on the average about 10 %). In computing the values of e given in Table 3 the corrections to the mean amplitudes for the observed mean frequency were applied.

The small, high frequency components of postural activity. In general, the high frequency components of postural activity were much more regular, both as regards period and amplitude, than the low frequency components on which they were superimposed. In the case of the trunk-mounted accelerometer, the tremor record indicated the actual high frequency movement of the trunk along an axis which was never far removed from the horizontal antero-posterior axis. Table 4 shows that the frequency varied from about 9 to 13 c.p.s. for the different subjects. The variation of the amplitude of acceleration between subjects was generally greater (1.6–5.9 cm./sec.²)—the difference between the two occasions for a particular subject was not so marked, and the mean of the values for the two occasions is therefore given in Table 4. The derivation of actual trunk displacement from trunk acceleration is comparatively simple, since the assumption of a sinusoidal nature for the

high frequency movements is reasonable. For a sinusoidal displacement the amplitude of acceleration will be $4\pi^2n^2$ (amplitude of displacement), where n is the frequency of both displacement and acceleration. The amplitude of trunk displacement at the high frequency will therefore be obtained if the acceleration amplitude is divided by $4\pi^2n^2$. Since $4\pi^2n^2$ has a value of the order of 5000, and the acceleration amplitude is of the order of 3 cm./sec.², it will be apparent that trunk displacement at the high frequency is very small in amplitude—about 0.0005 cm. or 5 μ . This is to be compared with a movement of the CG of the body with a mean amplitude at the low frequency of about 0.5 cm., so that the body displacement at the high frequency is only about one-thousandth of that which occurs at the low frequency.

The record obtained from the accelerometer mounted on the platform provided a measure of the high frequency component of the postural maintenance forces which were referred to the ground along the antero-posterior axis at the feet. The immediate origin of these forces is obviously the torque developed about the ankle axes during flexion and extension of the feet, though it could not be assumed that torques developed about joints (such as the hip joints) superior to the ankles would not also contribute to the forces of reaction developed between the feet and the platform. It seems likely, however, that the high frequency components of such torques from superior joints would be severely attenuated during transmission to the ground via the jointed skeleton. The frequencies and platform acceleration amplitudes obtained by analysis of records from the platform mounted accelerometer are summarized in Table 4. In this table the mean of the values obtained on the two occasions for each subject is given. The frequency of the tremor component at the feet was generally higher than that for the corresponding tremor component of trunk displacement, the mean value for all subjects being 15.4 c.p.s., compared with the mean value of 11.4 c.p.s. for trunk displacement tremor. There is no obvious explanation for this difference. It is also apparent from Table 4 that a subject with a relatively high tremor frequency at the feet did not necessarily have a relatively high frequency of trunk tremor. The amplitude of the high frequency component of the reactionary force at the feet could be derived from the platform acceleration amplitudes, since the horizontal deflexion of the platform per unit force was known from static calibration (17 μ /kg. wt.). The force amplitude was obtained by dividing the acceleration amplitude by $4\pi^2n^2z$, where n was the tremor frequency and z the calibration factor. The deduced force amplitude varied widely for different subjects and occasions (6.1–173.6 g. wt.), but subjects showed some consistency on the two occasions of examination, suggesting that the amount of this force tremor was to some extent characteristic of the subject. Another interesting feature which will be apparent from Table 4 was that a large force at the feet was usually but not always associated with a large displacement amplitude measured at trunk level (for example, Subjects H and J). However, the deduced force amplitude of the high frequency component at the feet was surprisingly small (average for all subjects and occasions of 26.3 g. wt.). Analysis of the low frequency component of the antero-posterior force at the feet (Figs. 1 and 3) was not carried out for this investigation, since no simple relationship between this force record and the records of antero-posterior body movement was apparent. It will, however, be apparent from the sample records reproduced in Figs. 1 and 3 that the amplitude of

the low frequency forces at the feet was appreciably greater than that of the analysed high frequency forces. This result is not in agreement with Smith (1957), who reported that the high frequency component of torque about the ankle joint was some 25 % greater than the amplitude of the low frequency component. The small values obtained with the platform technique for the force amplitude of the tremor also argues against the possible criticism that the displacement tremor recorded at trunk level was predominantly due to the actual horizontal displacement of the platform, for the actual measured displacement tremor of the trunk has a mean value of 6.2μ whereas the platform displacement corresponding to the mean force amplitude of 26.3 g. wt. would have been only (0.0263×17) or 0.45μ . Even the largest low frequency forces exerted antero-posteriorly at the feet, possibly of the order of 200 g. wt., would have given rise to platform displacements less than 5μ . There appears to be no evidence to suggest that displacements of this small extent, applied to the standing body via the soles of the feet, would be adequate to initiate actual reflexes in the human subject. It may therefore be presumed that the results discussed, obtained for a subject standing on the platform, are representative of normal standing on firm ground. This presumption is also confirmed by the similarity of the records obtained from an accelerometer mounted on the trunk for a subject standing either on the platform or on a normal floor.

DISCUSSION

The methods suggested by this investigation for describing postural movements during normal standing appear to be more objective than those which have been used by previous workers. In particular, the demonstration of non-rigidity of the body between trunk and lower limbs limits the significance of methods recording movement of the head only (e.g. Miles, 1950), and of biomechanical deductions which assume body rigidity (Smith, 1957). Where movements of the centre of foot pressure are used to define the movements of the body, greater precision is obviously obtained if the frequency and average magnitude of these movements are derived instead of the simple statement (e.g. Hellebrandt, 1938) of the limits of the movement. Such additional precision is obviously desirable if the methods are applied to a determination of the effects on posture of such practical situations as the carriage loads in the hands or on the back. The methods described would, of course, be equally adaptable to the investigation of sitting as well as of standing postures.

It may be suggested that a complete frequency analysis of the complex wave-forms characteristic of records of postural activity is necessary, and such analysis has been applied to body movements in man (e.g. Halliday & Redfearn, 1956). The use of this method implies the presence of a continuous spectrum of frequencies from the lowest to the highest. For practical purposes frequency analysis provides useful results if, and only if, the frequency components defined have some physical reality in the process under examination, and that the components can be presumed uniformly present in time. The low frequency elements of postural movement do not appear to obey this requirement, for the cycles of movement appear to vary, both as regards duration and amplitude, in a manner which can only be considered as random. Also, the discontinuity of the frequency spectrum of postural movements is suggested by virtual elimination of the low frequency elements by double differen-

tiation of the whole record (carried out, in the investigation described, by recording from the accelerometer attached to the platform). A frequency analysis of the high frequency elements of postural movement is perhaps more justifiable and could be applied if a more precise specification of this frequency band is required.

It is inconceivable that the postural movements described could have arisen, as suggested by the authors mentioned in the introduction to this paper, as pendular motion maintained over appreciable periods in a passive elastic system. Even for the low frequency movements, pendular motion would be feasible only if the centre of gravity of the mass moved symmetrically about the pivot, and all authorities agree that, for the antero-posterior movements of normal standing, the central position of the motion is a few cm. in front of the coronal plane including the ankle pivots. Pendular maintenance of the high frequency components of postural movement would require an order of passive elasticity much greater than is conceivable for the body tissues under stress during normal standing—particularly as a large proportion of these tissues would be muscle at a low level of contractile activity. Rough calculation shows that the bending elasticity of the lower parts of the body would have to be equivalent to that of a steel bar of 5 cm. square cross section for a body mass of 70 kg. to have pendular motion at 10 c.p.s. It must be concluded, therefore, that postural movements of the type recorded in this investigation are maintained by continuous muscular action. A similar conclusion was drawn by Smith (1957).

The origin of the high frequency tremor which appears to accompany all muscular activity has been the subject of much recent discussion. Some investigators have considered the tremor to arise from the servo-instability of the general stretch reflex mechanism (Halliday & Redfearn, 1956; Lippold, Redfearn & Vuco, 1957). Smith (1957), by inference from the electromyographic observations of Denny-Brown & Nevin (1941) and Denny-Brown (1949), suggests that the tremor arises from the graded recruitment of the motor units of the postural, anti-gravity muscles at 5–10 c.p.s. Marshall & Walsh (1956) considered that muscle, in responding to high frequency activation by nervous impulses, acts as a mechanical low pass filter to this activation. One of the present writers has suggested that the activation of the numerous constituent muscle fibres of a muscle via its nervous supply must proceed in a statistical manner, and temporal characteristics of activation of the individual fibre will be superimposed, as a tremor, on the resultant activity of the whole muscle (Whitney, 1958*b*). Since the basic temporal characteristic of muscle fibre contraction is a twitch-time of the order of 0.1 sec. (for mammalian postural muscle), it would be expected that a tremor frequency of the order of $1/(0.1)$ (or 10 c.p.s.) would be superimposed on all resultant activity of the postural musculature.

Little can be concluded from the present investigation of normal standing on a functional relationship between the high and low frequency components of postural activity. It is clear from the results presented, even for the small number of subjects involved, that a particular degree of low frequency movement may be associated with a very variable degree of high frequency tremor.

SUMMARY

1. Observations of antero-posterior postural sway during normal standing for 4-minute periods have been carried out on ten normal young males. A force analysis platform was used to record movements of the centre of foot pressure and the horizontal forces of reaction at the feet. An accelerometer mounted on the trunk was used to obtain a simultaneous record of the movements of the upper parts of the body.

2. All forms of postural activity investigated were cyclical in nature and included high and low frequency components. In the case of forces of reaction at the feet, effective separation of the high from the low frequency bands was possible

3. The low frequency components of movement of the centre of foot pressure and that of the change of trunk inclination was always very irregular, both as regards duration and amplitude of a complete cycle of movement. A method of analysis was therefore used to specify the average frequency and amplitude of the movement during the standing period. The average frequency of both movements varied, chiefly between the subjects, from 0.12 to 0.39 c.p.s.

4. The average amplitude of the low frequency movements of the centre of foot pressure varied, chiefly between subjects, from 0.36 to 1.13 cm. The average amplitude for a period of standing was about one quarter of the maximum limits of movement recorded for the period, and this suggests that the amplitude varies from cycle to cycle in a random manner. For dynamic reasons, movements of the centre of foot pressure must exaggerate the accompanying movements of centre of gravity of the body mass, the degree of the exaggeration increasing with increase of the frequency of the movement.

5. The low frequency component of change of trunk inclination had an average amplitude which varied, chiefly between subjects, from 0.54 to 1.33 (in units of 0.01 radian, 0.01 radian being equivalent to 0.573° of arc). Changes of trunk inclination were generally synchronized with movements of the centre of foot pressure, but the changes of inclination were typically much greater than would be expected if the body remained effectively rigid above the ankle joint pivots during antero-posterior sway. The results indicate that the trunk rotates relative to the limbs during standing, the axis of rotation being probably approximately coincident with the axis through the hip joints. The deduced relative movement at the ankle axis and at the 'hip joint' axis varied for the different subjects, and even for the same subject on the two occasions of examination, but the general result was that the rotations of lower limb about the ankle and of trunk about lower limb were about equal, and in the same direction.

6. The high frequency component of trunk displacement showed greater regularity, both as regards cycle duration and amplitude, than the corresponding low frequency component. The frequency of the displacement varied, chiefly between subjects, from 9.2 to 13.4 c.p.s. The average amplitude of the displacements at these frequencies varied from 2.8 to 15.0 μ , or about one-thousandth of corresponding low frequency displacements of the trunk at umbilical level as indicated by the movements of the centre of foot pressure. There appeared to be no simple relationship, however, between the variations of the two components for different subjects.

7. The high frequency component of the antero-posterior reactionary force at the feet has an average amplitude which varied widely between the different subjects (6.1–173.6 g. wt.). The frequency of this force component was somewhat higher (11.3–19.0 c.p.s. for the different subjects and occasions) than the corresponding frequency of trunk displacement.

8. The utility of the methods described for the objective assessment of postural activity, and the possible significance of the results obtained, are briefly discussed.

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SQUATTING FACETS ON THE TALUS AND TIBIA IN INDIANS

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The presence of so-called squatting facets on the upper surface of the neck of the talus, and on the corresponding part of the anterior margin of the lower end of the tibia, was first described by Thomson (1889). These facets have subsequently been studied by a number of workers. Several distinct facets have been described and, as Barnett (1954) has pointed out, lack of an agreed terminology has resulted in considerable confusion. Further, some workers have studied only tali, and others only tibiae. That results thus obtained are not strictly comparable is shown below. Finally, though there are several reports on adult bones, only one series of foetal tali has been reported (Barnett).

The present paper analyses critically the facets described by various authors, and classifies them (as far as possible) according to the terminology presented by Barnett. The results of a study of a series of adult and foetal Indian tali and tibiae are reported. The theory that the presence of the facets both in the foetus and the adult represents an example of the transmission of an acquired character to the offspring (Charles, 1894; Wood Jones, 1949) is discussed in the light of the findings.

MATERIAL

The material used for this study is as follows:

	Tibiae	Tali
Adult: dry bones	200	200
wet, cartilage-covered specimens	92	100
Foetal	66	66

In the case of the dry bones, while it was not possible to assign individual tali and tibiae to one another, they presumably belonged, in large part, to the same group of cadavers. The wet specimens were either freshly dissected or preserved museum specimens. In eight of these specimens the talus alone was available. The foetal specimens were from preserved foetuses varying from 112 to 234 mm. C.R. length. Specimens from the younger foetuses were examined under a stereoscopic microscope.

OBSERVATIONS

A. Forward prolongation and displacement of the medial articular surface of the talus

The medial articular surface of the talus is prolonged forwards beyond the level of the anterior margin of the trochlear surface in 188 out of 200 dry tali, in all the 100 wet tali, and in all the 66 foetal tali studied. The degree of forward prolongation in the dry tali is analysed in Table 6.

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Forward prolongation of the anterior end of the medial surface is usually accompanied, both in the adult and in the foetus, by a corresponding anterior displacement of the posterior end (Table 1).

The medial articular surface of the talus is frequently curved medially at its anterior end, in both foetal and adult specimens. The degree of curvature is very variable (Table 2).

Medial curvature and forward prolongation of the medial articular surface frequently coexist, but may be present independently (Table 3).

Table 1. *Forward displacement of the medial articular surface of the talus*

	Adult	Foetal
Forward displacement of both anterior and posterior ends	272	66
Forward displacement of anterior end only	16	—
Forward displacement of posterior end only	12	—
Total number examined	300	66



Fig. 1. Talus showing marked forward displacement and medial curvature of the anterior part of the medial articular surface.

Table 2. *Curvature medially of the anterior part of the medial articular surface of the talus*

Degree of curvature	Adult	Foetal
No curvature	88	12
Perceptible	59	10
Distinct	112	42
Marked	41	2
Total number examined	300	66

Table 3. *Relationship of medial curvature to forward prolongation of the medial articular surface of the talus*

	Adult	Foetal
Forward prolongation and medial curvature	204	54
Forward prolongation alone	84	12
Medial curvature alone	8	0
Neither present	4	0
Total number examined	300	66

Examination of dissected specimens shows that the anterior part of the medial articular surface, and the corresponding part of the malleolar facet are in perfect apposition only when the joint is strongly dorsiflexed.

B. *Medial extension of the trochlear surface of the talus*

The medial part of the trochlear surface is often prolonged anteriorly on to the neck of the talus. This area varies in size and shape (Figs. 2, 4 and 7). In some cases it is partially cut off from the trochlear surface by a notch and appears to be an almost distinct facet (Fig. 7). However, it always continues the antero-posterior curve of the trochlear surface. It comes into contact, in marked dorsiflexion of the ankle, with the undersurface of the lower end of the tibia and not with its anterior margin. A medial extension is seen in 165 out of 300 adult tali (55 %), and in 34 out of 66 foetal tali (51·5 %) examined.



Fig. 2. Talus showing a medial extension of the trochlear surface. See also Figs. 4 and 7.

Table 4. *Relationship of forward prolongation of the medial articular surface of the talus to medial extension of the trochlear surface*

	Adult	Foetal
Forward prolongation and medial extension	165	34
Forward prolongation alone	123	32
Neither	12	0
Total number examined	300	66

Medial extension of the trochlear surface is always accompanied by forward prolongation of the medial articular surface of the talus. However, the reverse is not invariably true (Table 4).

C. *Lateral extension of the trochlear surface of the talus*

The anterior margin of the trochlear surface is not straight but shows a convexity forwards. The forward convexity usually involves the lateral half to two-thirds of the trochlear surface, and its apex is often more central than lateral. In some of those specimens in which a medial extension is absent, the entire width of the trochlear surface shows a convexity forwards (Figs. 3, 4, 6 and 7). A lateral extension is present in 164 out of 300 adult tali (54·6 %), and in 43 out of 66 foetal tali (65 %) studied.

Examination of dissected specimens shows that both medial and lateral extensions of the trochlear surface are accompanied by corresponding changes in the shape of the articular surface of the tibia. However, articulation with the tibia occurred only at the extreme limit of dorsiflexion.



Fig. 3



Fig. 4

Fig. 3. Talus showing lateral extension of the trochlear surface. See also Figs. 4, 6 and 7.

Fig. 4. Talus showing both medial and lateral extensions of the trochlear surface.

D. Lateral squatting facet

This facet lies on the upper surface of the neck of the talus, usually towards the lateral side. However, it is sometimes on the centre of the neck, specially in foetal specimens (Fig. 8). It varies in size and shape (Figs. 5-7). It may be directly continuous with the trochlear surface or may be separated from it by a definite margin. Rarely, a non-articular strip separates it from the trochlear surface.

A lateral facet is distinguishable from a lateral extension by the fact that, whereas the lateral extension continues the antero-posterior curve of the trochlea the lateral facet does not. Thus while a lateral extension faces upwards and slightly forwards, a lateral facet faces upwards and, occasionally, slightly backwards. The antero-posterior diameter of a lateral extension is convex upwards, but that of a lateral facet is usually concave. These differences are obviously associated with the fact that it is only the true lateral facet that articulates with the anterior margin of the tibia, the extension articulating only with the undersurface of that bone. Lateral squatting facets are present in 86 out of 300 adult tali (28.6%) and in 8 out of 66 foetal tali (12%) examined.

A facet on the anterior margin of the tibia is not always accompanied by a facet on the talus. The facet is seen in 142 out of 200 dry tibiae examined, but is present in only 48 out of 200 dry tali. Further, in 92 dissections the facet is present on the tibia alone in 46 cases, and on both talus and tibia in 38 cases. While the tibial facet is less marked in the former group, it is nevertheless distinct. The facet on the talus is often smaller than that on the tibia, coming in contact with only part of the latter (Fig. 9).

Out of the 46 specimens showing facets on the tibia alone, 22 show a smooth area, not covered with cartilage, on the neck of the talus. This area comes into contact with the tibial facet in extreme dorsiflexion. In some of the remaining cases, the capsule of the ankle joint is attached fairly close to the margin of the trochlear surface and the tibial facet comes into contact with its lowermost part. In extreme dorsiflexion this part of the capsule is caught between the anterior margin of the tibia and the extra-articular part of the neck of the talus.

Out of 66 foetal specimens examined, 8 show facets on both bones, and 29 show a facet on the tibia alone.



Fig. 5

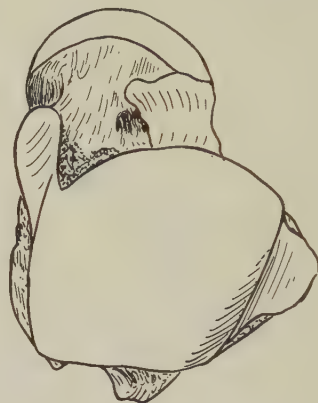


Fig. 6



Fig. 7



Fig. 8

Fig. 5. Talus showing a lateral squatting facet.

Fig. 6. Talus showing a lateral squatting facet and lateral extension of the trochlear surface.

Fig. 7. Talus showing a lateral squatting facet, and medial and lateral extensions of the trochlear surface.

Fig. 8. Talus of a foetus (175 mm. C.R. length) showing a large squatting facet covering almost the entire upper surface of the neck. A medial extension of the trochlear surface is also seen.

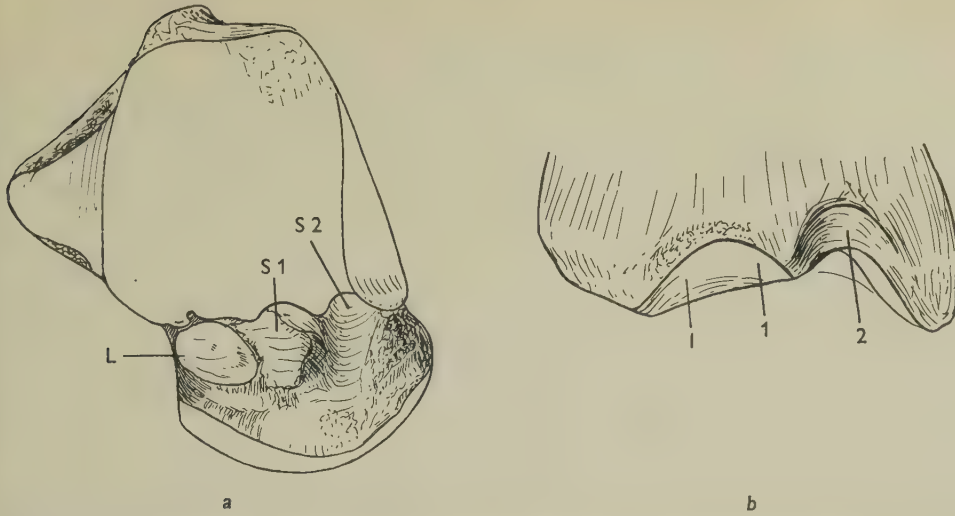


Fig. 9. (a) Upper surface of the talus showing a lateral cartilage-covered squatting facet (*L*) and two smooth areas, *S1* and *S2*. (b) Anterior margin of the tibia of the same specimen showing a large facet. Its lateral part (*l*) articulates with the facet on the talus; its medial part (*1*) with the area *S1* of the talus. More medially the tibia shows a smooth groove (*2*) which articulates with the smooth ridge *S2* on the talus.

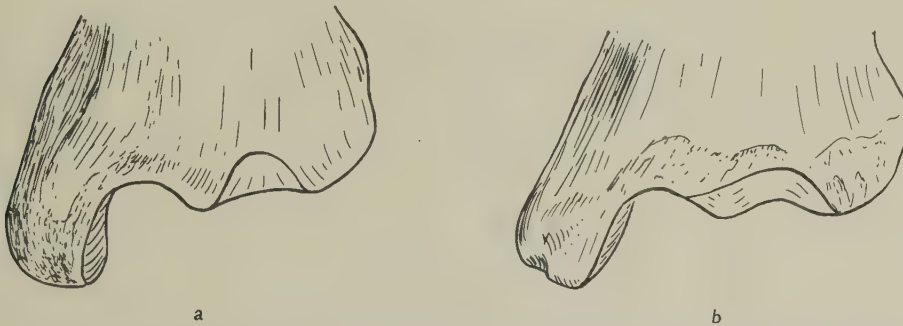


Fig. 10. Anterior surface of the lower end of the tibia showing two forms of the lateral squatting facet.

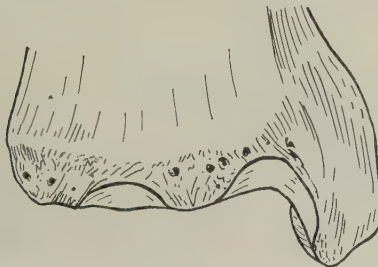


Fig. 11. Anterior surface of the lower end of the tibia showing distinct medial and lateral squatting facets.

E. *Medial squatting facet*

In the present investigation no distinct example of a facet on the medial part of the upper surface of the neck of the talus has been seen. In one dry specimen the medial extension of the trochlear surface does not continue the line of the trochlea but is concave upwards. Unfortunately, the tibia is not available for comparison, and hence it is not possible to say whether this area articulated with the anterior margin or with the undersurface of that bone. In five dry tibiae, a distinct facet is seen on the medial part of the anterior margin, separated from the lateral facet by a non-articular area.

F. *Relationship of the various modifications associated with squatting to one another*

A description of the modifications due to squatting would be incomplete without an analysis of the relationship that they bear to one another (Table 5).

Table 5. *Relationship of the various modifications of the talus, associated with squatting, to one another*

Modification present	Adult	Foetal
Medial extension alone	59	9
Lateral extension alone	40	18
Lateral facet alone	12	0
Medial and lateral extensions	65	19
Lateral extension and lateral facet	33	2
Medial extension and lateral facet	15	2
Medial and lateral extensions and lateral facet	26	4
No modification	50	12
Total number examined	300	66

DISCUSSION

A. *Forward prolongation and displacement of the medial articular surface of the talus*

A comparison of the findings in dry bones of the present series with those of Barnett (Table 6) shows that forward prolongation of the medial articular surface of the talus is much more pronounced in Indian bones than in those of Europeans.

Table 6. *Forward prolongation of the medial articular surface of the talus*

Percentage of the antero-posterior diameter of the medial articular surface extending beyond the anterior margin of the trochlea	European tali (Barnett) (%)	Indian tali (present series) (%)
0-10	48	7
11-20	34	15.5
21-30	17	31
31-40	1	34
41-50	0	12
Above 50	0	0.5

Forward prolongation of the medial articular surface is, as shown above, usually accompanied by a forward displacement of the posterior end. Barnett observed this association in the European foetus, but not in the adult.

Although Barnett mentions the presence of medial curvature of the anterior part of the medial articular surface, he does not say how frequent and how marked it is. Comparison of his findings in the European with those of the present series is, therefore, not possible.

Charles (1893), who first described these features, attributed them not only to squatting, but also to the so-called sartorial position. According to him, in this position there is plantar flexion and marked adduction at the ankle. He claims that these modifications of the talar medial articular surface render the adoption of this posture easier. As shown above, however, the anterior parts of the medial articular surface and the corresponding part of the malleolar facet are in perfect apposition only when the joint is strongly dorsiflexed. This clearly shows that this modification is related to the marked dorsiflexion associated with squatting, and has no relation to the sartorial position. This is further confirmed by the fact that forward prolongation of the medial articular surface may be present even in people who possess marked mobility of the ankle joint (Wood Jones 1949), but who never adopt the sartorial posture.

B. Medial extension of the trochlear surface of the talus

The fact that a medial extension comes into contact, in marked dorsiflexion of the ankle, with the undersurface of the lower end of the tibia and not with its anterior margin, shows that it is not a true squatting facet. This agrees with the view expressed by Inkster (1927), and Barnett. The so-called medial facets described by Parker & Shattock (1884), Charles (1893), and Sewell (1904), are all found, on critical study of their texts and figures, to be merely extensions of the trochlear surface and

Table 7. *Medial extension of the trochlear surface of the talus*

	Author	Race	Total no. studied	Medial extension present	Percentage
Adult	Charles	Punjabi	53	25	47.2
	Sewell	Egyptian	1006	189	19
	Barnett	European	100	11	11
	Present series	Indian	300	165	55
Foetal	Barnett	European	56	44	78.5
	Present series	Indian	66	34	51.5

not true squatting facets. This is further confirmed by the fact that the incidence of the medial facet described by Charles in the Punjabi (47.2 %) corresponds fairly closely to incidence of medial extension of the trochlear surface (55 %) observed, in material collected from the same region, in the present series. The incidence of medial extension of the trochlear surface found in the present series is compared with findings of other workers in Table 7.

C. Lateral extension of the trochlear surface of the talus

The incidence of lateral extension of the trochlear surface in bones of the present series is compared with that in European bones (Barnett) in Table 8.

Table 8. *Lateral extension of the trochlear surface of the talus*

	Author	Race	Total no. studied	Lateral extension present	Percentage
Adult	Barnett	European	100	17	17
	Present series	Indian	300	164	54.6
Foetal	Barnett	European	56	Common	?
	Present series	Indian	66	43	65

D. *Lateral squatting facet*

Earlier workers (Thomson, Charles, Sewell) did not distinguish the lateral extension of the trochlear surface from the lateral squatting facet. It is possible that some of the instances of lateral facets described by them were in reality merely lateral extensions. It might be argued that the absence of a corresponding facet on the tibia would always enable a lateral extension to be correctly distinguished from a true facet. That this is not so is clear from the fact that, as shown above, facets are very often present on the tibia, unaccompanied by any definite facet on the talus. This fact was clearly brought out by Thomson, but seems to have been overlooked by subsequent workers. Thus a lateral extension is easily confused with a facet unless the criteria put forward above (p. 543) have been kept in mind. Considering that Charles studied material collected from the same region as in the present investigation, the much higher incidence of the lateral facet found by him supports this contention. The incidence of lateral facets on the talus and tibia in the present series is compared with that found by other workers in Tables 9 and 10 respectively.

Table 9. *Lateral squatting facet on the talus*

	Race	Author	Total no. studied	Lateral facet present	Percentage
Adult	European	Thomson	25	1	4
		Pfitzner	840	1	—
		Barnett	100	2	2
	Australian	Thomson	11	7	63.6
		Inkster	150	45	30
	Egyptian	Sewell	1006	86	8.6
	Indian	Charles	53	34	64
		Present series	300	86	28.6
Foetal	European	Barnett	56	13	23
	Indian	Present series	66	8	12

Table 10. *Squatting facet on the tibia*

Race	Author	Total no. studied	Lateral facet present	Medial facet present
European	Thomson	30	2 (6.6%)	—
	Wood	118	20 (17%)	2 (1.7%)
Australian	Thomson	14	11 (78.5%)	—
	Wood	236	190 (80.5%)	5 (2.1%)
Indian	Charles	52	45 (86.5%)	9 (19.2%)
	Present series	292	226 (77.4%)	5 (1.7%)

E. *Medial squatting facet*

The medial squatting facet described by Barnett as an area on the medial part of the upper surface of the neck of the talus, not continuous with the trochlear surface, and not articulating with the tibia, is a dubious entity. If it does not articulate with the tibia, there is no justification in including it among squatting facets. It has been shown above that most of the so-called medial facets described by Parker & Shattock, Charles, and Sewell, are really medial extensions of the trochlear surface. A medial facet on the anterior margin of the lower end of the tibia has been described by Charles and by Wood (1920), and has also been seen in the present series. Charles claims to have seen specimens in which this facet articulates with a facet on the neck of the talus, but does not say how often this was seen. A true medial facet on the talus, if present, must indeed be rare considering that no other worker describes it. None is seen in the present series. That facets on the tibia are not necessarily accompanied by facets on the talus is clear from what has been said about the lateral squatting facet.

F. *Inheritance of acquired characters*

The fact that modifications in bones attributed to squatting are to be seen not only in the adult, but also in the foetus, led Charles (1894) to believe that these modifications, acquired by the individual, had in course of time become an inherited characteristic of the Punjabi. However, the presence of these features in the European foetus (Sewell, Barnett) can hardly be explained on this hypothesis. A consideration of Table 11, comparing the findings of Barnett in the European adult and foetus with those of the present series, clearly shows that the modifications associated with squatting are, on the whole, more frequent:

- (i) in the Indian adult as compared to the European;
- (ii) in the European foetus as compared to the adult;
- (iii) in the Indian adult as compared to the foetus; and
- (iv) in the European foetus as compared to the Indian.

Table 11. *Percentage squatting facets in European and Indian tali*

	Adult		Foetal	
	European	Indian	European	Indian
Medial extension	11	55	78.5	51.5
Lateral extension	17	54.6	Common	65
Lateral facet	2	28.6	23	12

From the above the following conclusions are obvious:

(a) That the Indian inherits no greater tendency to the development of squatting facets than does the European.

(b) That these features present in the European foetus tend to disappear in the adult. This lends strong support to the view expressed by Sewell that the presence of these features in the foetus is simply due to the fact that 'during intra-uterine life the lower extremities of the foetus are in the position most favourable for the formation of such articular surfaces, viz., one of extreme dorsiflexion and inversion'.

(c) The fact that these features persist in, and even undergo further development in, the Indian adult can accordingly be attributed to the fact that in squatting he continues to maintain the dorsiflexed position for considerable periods.

(d) That the presence of 'squatting' facets both in the foetus and adult in Indians is a mere coincidence and is not indicative of the fact that these features have been inherited.

SUMMARY

1. The so-called squatting facets described in the literature have been critically analysed.

2. The incidence of these facets in a series of 300 adult and 66 foetal tali, and in 292 adult and 66 foetal tibiae, is described.

3. Modifications produced by squatting are more frequent in (a) the Indian adult than in the European, (b) the European foetus than in the Indian, (c) the Indian adult than in the foetus, and (d) the European foetus than in the adult.

4. It is concluded that these facets in the Indian adult are purely acquired and are not inherited.

I am indebted to Dr Indarjit, Medical College, Amritsar, and to Dr G. N. Constable, Christian Medical College, Ludhiana, India, for permission to examine some material in their departments.

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'SPURT' AND 'SHUNT' MUSCLES: AN ELECTROMYOGRAPHIC CONFIRMATION

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INTRODUCTION

By the application of mathematical analysis MacConaill (1946, 1949) has shown that skeletal muscles act as 'shunt' or 'spurt' muscles. However, there has been a lack of experimental data to confirm or disprove his theories. Examination of the findings of certain of our electromyographic experiments on the flexors of the elbow joint (performed with other aims in mind) appear now strongly to confirm MacConaill's calculations and conclusions.

MATERIAL AND METHODS

Material and methods have been described in a previous paper (Basmajian & Latif, 1957) concerned with a broader analysis of the actions of the elbow-flexors. The findings made during that investigation which are significant to the present study are considered here. For a detailed description of the apparatus used see Basmajian (1958).

FINDINGS

Flexion of the elbow. During slow flexion of the elbow, with or without a load of two pounds, the brachioradialis was relatively quiescent in most of the twenty subjects, while the biceps and the brachialis showed considerable activity. On the other hand, with quick flexion of the elbow the brachioradialis became very active in almost all the subjects.

Maintenance of flexed posture. During maintenance of flexed postures against the force of gravity, the biceps and the brachialis were almost always active while the brachioradialis was either inactive or only slightly active. Even the addition of a load of two pounds made little change in the activity of the brachioradialis.

Extension of the elbow. During slow extension there was some slight activity in all of the muscles acting against the force of gravity with or without the added load. With quick extension, there was a general increase of activity, that in the brachioradialis being most pronounced.

DISCUSSION

On the basis of MacConaill's theory, the biceps and brachialis are chiefly spurt muscles at the elbow while the brachioradialis is chiefly a shunt muscle. In other words, except in complete extension, the former two muscles act mainly across the long axis of the forearm providing the acceleration along the curve of motion (Fig. 1). The brachioradialis, on the other hand, remaining more or less parallel to the forearm throughout the range of motion, acts mainly along the long axis of the

forearm to provide the centripetal or shunt force and the required stabilization at the elbow joint (MacConaill, 1949).

Obviously, when the joint is not moving, the muscular forces along the bone and through the joint are equal to the total load of the limb. We know from common experience, confirmed by electromyography, that with no added weight and the limb hanging free this force is minimal. The load here is the weight of the limb beyond the elbow joint and the ligaments alone are adequate to carry it. The addition of a weight held in the hand increases the muscular activity in the biceps and the brachioradialis.

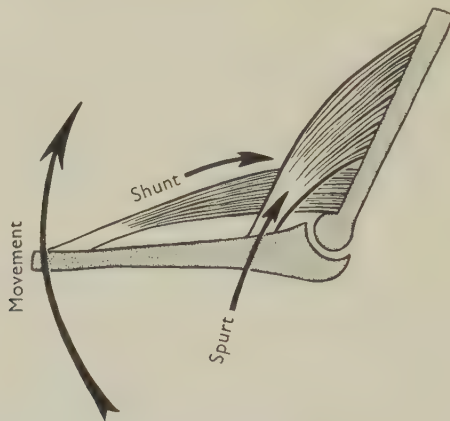


Fig. 1. Schematic drawing of the direction of action of a shunt muscle (along the bone) and of a spurt muscle (across the bone). (Modified after MacConaill.)

During very *slow* uniform flexion of the elbow joint, the shunt forces along the forearm will be approximately unchanging. Thus no great increase in the activity is required from the muscles for shunt or centripetal force. But if the flexion is rapid, a greater shunt or centripetal force *is* required. That force cannot be provided by the spurt muscles because they would impart a centrifugal acceleration along the tangent to the curve instead of producing a uniform rapid movement. Therefore the shunt muscles are called upon—indeed, must be called upon—for much greater activity. Our electromyographic findings confirm this. The brachioradialis, the typical shunt muscle, shows its greatest activity during uniform quick flexion of the elbow. During slow flexion and during the maintenance of flexed postures, it showed little or no activity even with a load of two pounds in the hand.

Our experiments have shown that during both slow and quick extension of the elbow all the flexor muscles show considerable activity. During slow extension the 'letting-out' function of the antagonist muscles is called upon. However, during quick extension there would seem to be a need for complete inhibition of the antagonists. Experimentally, this did not occur. Barnett & Harding (1955) concluded from similar findings with the biceps alone that the antagonists come into strong contraction at the end of a whip-like movement due to the stretch reflex. It would appear that this protects the joint which otherwise would be injured.

In the earlier paper referred to (Basmajian & Latif, 1957) it is stated that during extension a short, sharp burst of activity is found in all three muscles, biceps,

brachialis and brachioradialis, and that it is most pronounced in the biceps. In reviewing the records it was found that the brachioradialis in general was more active than biceps or brachialis during quick extension, providing further confirmation of MacConaill's mathematical theory. The basic requirement for a shunt muscle is to provide centripetal force during rapid movement in the circular path and so the direction of movement (regardless of whether it is in the direction of flexion or extension) is of no consequence.

SUMMARY

The mathematical theory and calculations by MacConaill by which he demonstrated the existence of 'shunt' and 'spurt' activity in skeletal muscles have been confirmed by the electromyographic findings for the elbow-flexors in a series of normal persons.

The brachioradialis is a good example of a shunt muscle. Generally inactive during static postures and slow movements, it is very active during both quick flexion and quick extension as MacConaill's theory requires. This is because the direction of movement is of no consequence when a muscle's chief function is to provide centripetal force.

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REVIEWS

The Hand: Its Anatomy and Diseases. By JOHN J. BYRNE, M.D. (Pp. xiv + 384; 166 illustrations; 80s.) Oxford: Blackwell Scientific Publications.

Simple pentadactylous manus though it may be, the human hand, as the late Prof. Wood Jones pointed out, has never lacked its champions. With this volume Prof. Byrne, well-armed at every point, enters the lists.

Following a straightforward and business-like account of the anatomy and development of the hand, successive topics dealt with are infection, trauma, other diseases, and finally reconstructive surgery. The sequence is logical, the presentation clear and the scope comprehensive; for it may be doubted if there is a hand condition other than those which fall solely within the province of the dermatologist which has escaped the author's notice. Nor is his account a mere catalogue: against the background of his own experience the findings of other workers in the field—neuro-, plastic and orthopaedic surgeons—are fairly discussed and full references given.

The book of course is written for clinicians to whom its merits will be obvious enough, and the reviewer is confident that those of them who make a copy their own will feel well rewarded. But what of anatomists? Strictly, only the first sixty pages are of direct anatomical interest and, truth to say, their content is not unfamiliar. However, in the succeeding chapters there is scarcely a page which does not contain some fact or other by which students' interest might be quickened and their understanding of topographical anatomy enriched. Some of these, such as the occupational hazard of gamekeeper's thumb or the visualization of the mid-palmar space by accidental injection of compressed air, are odd enough to be sure; and without pointing a moral it would appear that whatever mischief idle hands may find to do there seems hardly a danger which busy hands may not encounter.

Misprints in the text are few and not such as to cause trouble. The illustrations have been well chosen and nearly all have reproduced satisfactorily, although in future editions one or two of the X-rays might be replaced by others showing better contrast. On p. 38 the account given of the muscular distribution of the radial nerve is somewhat different from usual; on p. 41 space might have been found for some reference to the supratrochlear lymph node; and on p. 153 the role of the Schwann cell in nerve regeneration is not afforded the importance which it deserves.

This is certainly a meagre list of criticisms in a work of close on 400 pages, for the fact is Prof. Byrne has produced a very readable and useful book which should find a place in all medical school libraries.

E. W. WALLS

The Effect of Advancing Age upon the Human Spinal Cord. By L. RAYMOND MORRISON with the collaboration of S. COBB and W. BAUER. (Pp. x + 127; 48s.) Harvard University Press. London: Oxford University Press. 1959.

This book is a study of autopsy specimens from thirty-one subjects who died from non-neurological diseases between the second and ninth decades of their lives. In addition, there is an atlas of a 'normal' spinal cord removed from the body of a woman who died from subacute bacterial endocarditis at the age of twenty-four.

The authors endeavour to relate the histological differences found in these cords to age; they are fully aware that some of these differences may be related to the systemic diseases from which the patients suffered. All the histological differences that were found are described even if they had no connexion with the ageing process. An attempt is then made to

separate these changes due to ageing by considering the 'more constant alterations that are concomitant with advancing age'.

It is found that there is a general thickening of the meninges with increasing age, the dura being more consistent than the pia-arachnoid which shows greater variation from decade to decade and even between different levels of the same cord. Myelin loss takes place with advancing age, especially in the posterior columns and to a lesser extent in the lateral cortico-spinal tract, but in one third of the cases studied there was no correlation between the myelin loss and the age group concerned. Myelin loss was also seen in the anterior roots.

Various kinds of degenerative processes were seen in the anterior horn cells including acute swelling, axonal reaction, shrinkage and pigmentary atrophy. These neurons and those of Clarke's column became increasingly chromatolysed with advancing age.

Microglia were found in all cases and were only slightly more numerous in old age than at any time after the middle decade since the myelin and axons degenerate at almost a steady rate which does not increase substantially in old age. Astrocytes proliferate throughout the white matter whenever there is a loss of myelin. Corpora amylacea, presumed to be derived from oligodendroglia, increase rapidly with advancing age and there is a general denser gliosis in the grey matter that is progressively found in the later age groups.

The authors summarize their conclusions in the words 'Lesions resulting from advancing age first begin as constant findings, about the fourth decade and, in a general way, increase in severity through the rest of life. These lesions involve all the structures of the cord and are widespread and often systematic.'

The atlas of the 'normal' cord shows illustrations of Weigert and Nissl stained sections taken at each segment of the cord and accompanied by short descriptions. In addition, counts of the numbers of normal nuclei in cubes of side 250 micra are given for the different columns. These were made by counting the nuclei in each column in ten consecutive sections. There is some doubt as to the utility of these counts since the variability between the numbers found for each side of the cord at the same level is often very considerable. Moreover, there are no figures available to show the variability between the counts for different cords at the same level.

This study gives an excellent and detailed account of the histological differences to be found in the spinal cord at different ages using a variety of staining techniques. The book is well produced and the illustrations are of a uniformly high standard.

D. A. SHOLL

Memoirs of the Society for Endocrinology No. 6: Implantation of Ova. Edited by

P. ECKSTEIN. (Pp. viii + 97; 30s.) Cambridge University Press. 1959.

The present volume records the Proceedings of a Conference held in November 1957 to survey the phases of mammalian development from the time of fertilization until the placenta has been established. The various factors—hormonal, biochemical and pharmacological—concerned in implantation and the maintenance of pregnancy are discussed in the light of recent investigations. A survey of the physiology of the implantation of the ovum in mammals is given by Dr Eckstein, Dr Shelesnyak and Prof. Amoroso. The morphological aspects of implantation are discussed by Prof. Amoroso who describes the attachment cone of the guinea-pig blastocyst as observed under time-lapse phase-contrast cinematography. The factors governing the spacing of implantations within the uterus are considered by Drs McLaren and Michie and delayed implantation by Prof. Harrison and Mr Neal. Dr Psychoyos gives an account of pseudopregnancy and deciduoma formation. The contributions on histochemical and biochemical topics are dealt with separately by Prof. Boyd, Dr Lutwak-Mann, Prof. Robson, Prof. Mayer and Dr Shelesnyak. In the discussions following each contribution, an opportunity was given to assess, compare and correlate the results of the various methods used in the elucidation of the relationship between ovum and endometrium.

The memoirs make a worth while contribution to the subject of nidation and should be on the book-shelf of every embryologist.

W. J. HAMILTON

Handbuch der mikroskopischen Anatomie des Menschen. By W. v. MÖLLENDORFF. Fortgeführt von W. BARGMANN. Bd IV, *Nervensystem*. Teil 3: *Sensible Ganglien*. By J. H. SCHARF. (Pp. viii + 485, 298 illustrations. Ladenpreis: Geheftet DM 198; Halbfranz, DM 216). Teil 8: *Das Kleinhirn*. Ergänzung zu Bd IV/1. By J. JANSEN and A. BRODAL. (Pp. viii + 323, 197 illustrations.) Ladenpreis: Geheftet, DM. 124; Ganzleinen. DM, 142). Berlin, Göttingen, Heidelberg: Springer Verlag. 1958.

These two volumes represent further authoritative additions to the von Möllendorff *Handbuch*. The section on the sensory ganglia is by Prof. Joachim-Hermann Scharf of the Anatomical Institute of the University of Jena. It is a very well illustrated and critical account of the development and structure of the cranial and spinal ganglia which could stand on its own as a monograph devoted to these structures. There is, to the reviewer's knowledge, nothing in the literature which deals so fully and so excellently with the field considered. The accounts of the embryology of the ganglia and of the cytology of their constituent cells are particularly valuable.

The volume on the cerebellum by Profs. Jansen and Brodal brings up to date the account of this part of the brain which appeared in the *Handbuch* thirty years ago under Jakob's name. As is to be expected from two authors who have themselves contributed so much to our information on the cerebellum, the present account is an authoritative one. In particular the descriptions of the connexions of cerebellum with other parts of the nervous system will be found most helpful.

In spite of their expense these two volumes will be essential for all workers on the structure of the nervous system. They are beautifully produced and possess excellent bibliographies.

J. D. BOYD

The Comparative Morphology of the Carotid Body and Carotid Sinus. By W. E. ADAMS. (Pp. xviii + 272; 52 illustrations + 5 plates; 80s.) Springfield, Illinois: Thomas. 1958.

This monograph by Prof. Adams can be most warmly recommended as a satisfactory presentation of a difficult and important morphological problem. The scattered and extensive literature on the carotid body and the carotid sinus has been surveyed in a most scholarly manner, and the author has taken great pains to see every significant contribution to our knowledge on these functionally important and highly specialized structures. Writing, as he does, from a part of the world where great reference libraries do not exist, the full coverage of a literature in many languages which goes back more than 200 years must in itself have been a major endeavour. But Prof. Adams has condensed his reading into a coherent account of the evolution of our knowledge on the significance of the region of bifurcation of the internal carotid artery and has added to this account a survey of his own important contributions. The result is a comprehensive critical analysis of present-day knowledge of the region concerned throughout the Vertebrata which will be invaluable to all who are interested in chemo- and pressor-receptors. It is to be regretted that the volume does not include a full survey of the carotid-body-like organs in the thorax and the afferent nerve-supply to other great vessels. But the absence of extension of the account to include these other comparable, and presumably homologous, structures, can hardly be taken to detract from the merit of the presentation. Prof. Adams's volume will be invaluable for those interested directly in the field concerned, and it will be an excellent introduction to that field for all biologists.

The volume is excellently produced and well illustrated: it possesses a fine bibliography and a helpful index.

J. D. BOYD

The Cell. Edited by JEAN BRACHET and ALFRED E. MIRSKY. Volume I. (Pp xxi + 816; illustrated; \$22.00.) New York: Academic Press. 1959.

This is the first of three volumes which collectively are intended to cover our present knowledge of the cell. It is an ambitious project and, apparently, an expensive one. This first volume is divided into two parts, the first concerned wholly with methodology, and the second with selected problems in cell biology. The thread connecting these two parts and the various articles in them is so tenuous that little can usefully be said of the book as an integrated unit and it seems better to review it as a series of separate articles by specialists in their respective fields of research.

Part I consists of eight articles which vary greatly in length and scope. Of the four shorter articles two, on microscopy and on quantitative histochemistry, merely give a broad survey of the types of techniques available; the third, by Ficq, is a particularly useful account of track autoradiography; and the last, by Kopac on micrurgical studies on living cells, will be of but theoretical interest to most research workers in this country for the apparatus described is well beyond the range of most equipment grants on this side of the Atlantic. A chapter on tissue culture techniques includes a general review of the problems that have been studied and could be studied by this type of experimental approach. Fixation and staining are dealt with by Gersch in an article which does less than justice to some of the more modern aspects of histochemistry. The two longest articles are particularly valuable. That by Walker & Richards is an excellent account of quantitative microscopical methods applicable to single cells. They discuss methods available for determination of total mass and of optical density (for both natural pigments and cytological stains) with a full explanation of the errors involved. That by Allfrey on the isolation of subcellular components is an excellent review of the methods available and should be most valuable to any non-biochemist wishing to utilize these traditionally biochemical techniques.

Part II consists of seven articles on specific problems of cell biology. The first of the articles, on fertilization, is concerned mainly with events in sea urchin eggs. The second article, on sex determination, gives a concise account of the various factors, genetic and otherwise, which determine the sex of an individual in both vertebrates and invertebrates. The third article, by Grobstein, deals with the very difficult subject of differentiation in vertebrate cells. It draws together data from a number of experimental fields and throws some refreshing new light on old problems. The next article, on cell growth and differentiation in plants, emphasizes the need for more research in this field with modern techniques. The article by Briggs & King on nucleo-cytoplasmic interactions during embryonic development reviews both the earlier literature and their own recent work on nuclear transplantation in amphibia. The next article attempts to summarize our present knowledge of transplantation immunity—a difficult article for the non-specialist. The final chapter is a concise, clearly written summary of our present knowledge of the effects of various types of radiation on living cells.

From the foregoing paragraphs it will be clear that this volume consists of a series of articles differing greatly in scope and style. One cannot help wondering for whom this book was written. Few people are likely to read it straight through from cover to cover (if they did they would find it an exacting task). Few people are likely to be interested in all the articles, most of which are too detailed for the general reader yet often not sufficiently so for the specialist in that particular field. Clearly it has a place in the biological library. Its place could be just as well filled, however, by a series of monographs each dealing with some specific facet of the cell—books small enough and homogeneous enough to take on a long railway journey or to read at the week-end.

There has in recent years been an increasing number of books of this type—a collection of review articles with slight relevance to each other. This trend, if it continues, may well do a disservice to science: certainly it will do little to advance either research or education. The fault lies not only with the publishers, whose idea it presumably is that such books are necessary, but also with the writers of many of the individual articles who do not take

sufficient pains to produce a balanced and readable account of the subject they have been asked to review. The remedy, one feels, lies in the hands of those who are asked to edit such projects.

P. R. LEWIS

The Cranial Nerves. Anatomy and Anatomico-Clinical Correlations. By ALF BRODAL. (Pp. 141 and 25 figures; 15s.) Oxford: Blackwell Scientific Publications. 1959.

This book gives a description of the peripheral course and central connexions of the cranial nerves, and surveys the various disorders of function which most commonly result from their involvement by disease processes. One might quarrel with the nerve to stapedius being included as a branch of the nervus intermedius, otherwise this part of the account follows accepted lines. The juxtaposition of facts should help the student to grasp the anatomical basis of clinical syndromes, although the requisite information is already not difficult to obtain from existing textbooks of anatomy and neurology. More stimulating in the field of neurological research are the passages which relate to comparatively unfamiliar matters, such as corticofugal inhibitory pathways and somatotopical arrangement within nuclei and tracts. It is a pity that recent work on these and other subjects should have been presented rather summarily, without a detailed account of the findings or much discussion of their significance. There is a bibliography to lead the reader to some of the salient papers; nevertheless, one would have appreciated a fuller and more critical treatment from so outstanding an authority. Within its limits, however, the exposition is concise and clear. A few errors in spelling, punctuation and syntax have crept into the translation.

C. C. D. SHUTE

BOOKS RECEIVED

- Biological and Biochemical Bases of Behaviour.* Edited by H. F. HARLOW and C. N. WOOLSEY, 1958. (Pp. xx+476. \$8.00.) Madison: The University of Wisconsin Press.
- An Atlas of Normal Radiographic Anatomy.* By I. MESCHAN, 2nd edition, 1959. (Pp. xxi+759. £5. 12s. 0d.) Philadelphia and London: W. B. Saunders Co.
- Angewandte und topographische Anatomie.* By G. TÖNDURY, 2nd edition, 1959. (Pp. xiii+578. Ganzleinen, DM. 79.) Stuttgart: Georg Thieme Verlag.
- Bauprinzipien des Säugerskeletes.* By B. KUMMER, 1959. (Pp. xi+236; 169 illustrations. Ganzleinen, DM. 45.) Stuttgart: Georg Thieme Verlag.
- The Scope of Physical Anthropology and its Place in Academic Studies.* A report of a Symposium held at the Ciba Foundation, 6 November 1957. Edited by D. F. ROBERTS and J. S. WEINER. 1958. (Pp. 66.) Published for the Society for the Study of Human Biology by the Wenner Gren Foundation.
- Cell, Organism and Milieu.* Edited by DOROTHEA RUDNICK, 1959. (Pp. v+352; 135 illustrations. \$8.00.) New York: The Ronald Press Co.
- A short Introduction to Anatomy. (Isogogae Breves.)* By JACOPO BERENGARIO DA CARPI. Translated with introduction and historical notes by L. R. LIND and anatomical notes by P. G. ROOTE. (Pp. xi+228; many facsimile illustrations. 37s. 6d.) Chicago: University of Chicago Press. London: Cambridge University Press.
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- Connective Tissue, Thrombosis, and Atherosclerosis.* Proceedings of a Conference held at Princeton, 1958. Edited by I. H. PAGE. (Pp. x+316. \$9.50.) New York and London: Academic Press.
- Recent Progress in Endocrinology of Reproduction.* Proceedings of a Conference held at Syracuse, 1958. Edited by C. W. LLOYD. (Pp. xi+532. \$12.00.) New York and London: Academic Press.

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

NOVEMBER 1958

The Annual General Meeting of the Society for the Session 1958-9 was held on Friday, 28 November and Saturday, 29 November 1958 in the Department of Anatomy, Guy's Hospital Medical School, London, S.E.1. During the various Sessions the Chair was occupied by the President (Prof. F. GOLDBY) and by Prof. R. WARWICK.

The following are the authors' abstracts of the papers presented.

Some effects of processing on myelinated nerve fibres. By P. L. WILLIAMS and C. P. WENDELL-SMITH. *Guy's Hospital Medical School, London*

Despite the frequency of quantitative observations on peripheral nerve using fixed-stained preparations, attempts to analyse the changed size relationships which result from the method of processing used have in general proved satisfactory. Using a technique described at a previous meeting, 5μ fresh frozen sections have been examined under the polarizing microscope and the size relations of the myelin sheath determined for muscle and cutaneous nerves of the rabbit. As considerable differences are found to exist between the two types, the present investigations are restricted to the nervus gastrocnemius medius. Observations on fresh nerve have been compared with measurements obtained from sections stained by Wolters' technique (*Z. wiss. Mikr.* 1890, 7, 466) following fixation in Flemming's fluid, and changes in the external/internal diameter ratio studied.

The changes in external diameter have been studied by comparing sections prepared by these two techniques from adjacent portions of the same nerve trunk. Some of the changes that occur when a fresh frozen section is subjected to the successive action of fixatives, dehydration and clearing agents are demonstrated.

Size relationships in teased nerve fibres. By C. P. WENDELL-SMITH and P. L. WILLIAMS. *Guy's Hospital Medical School, London*

In a previous communication we have noted that when fresh teased preparations of peripheral nerve are examined microscopically, relative measurements of internal and external sheath diameter are affected by the optical properties of the highly refractile myelin and of the immersion medium. A theoretical treatment involving a consideration of the appropriate refractive indices and the geometrical form of the interfaces, and the subsequent construction of scale diagrams is presented. Experiments have been performed in which the nervus gastrocnemius medius of the rabbit has been teased in fluids of varying refractive index, examined under conventional light and polarizing microscopes, and photographed.

In fluids of high refractive index the myelin sheath is not apparent, but the lower refractive index of the axoplasm renders it visible. In a fluid of slightly lower refractive index the sheath is visible and relatively thick. Thereafter, serial reduction of the refractive index is accompanied by a parallel reduction in apparent sheath thickness.

A detailed analysis of conditions obtaining when normal saline is used as the immersion medium has been made. The results are compared with those derived from a study of fresh-frozen and fixed-stained transverse sections.

Some observations on the mesencephalic nucleus of the trigeminal nerve.By ROGER WARWICK. *Guy's Hospital Medical School, London*

Deiters (1865) and Johnston (1909) have established the status of the mesencephalic nucleus of the trigeminal nerve as sensory, and the latter has demonstrated their equivalence to dorsal root ganglion cells. Views on the connexions and function of this nucleus have varied, but most observers have considered it to be concerned with proprioception. Tozer & Sherrington (1910) have thought that proprioceptor fibres from the eye muscles enter the brainstem by their motor nerves. Tozer (1912) has found section of these nerves without effect on the trigeminal midbrain nucleus, whereas division of the trigeminal trunk causes marked degeneration. Kosaka (1912) has concluded from section of the nerve's divisions that the majority of the axons of cells in this nucleus lie in the mandibular branch, a finding largely confirmed by Corbin (1940). He and his co-workers have concluded, from the results of destructive lesions and the recording of action potentials in the midbrain, that the mesencephalic nucleus is proprioceptor for masticatory but not ocular muscles. They have considered the ocular proprioceptor nerve endings to be innervated by cells in the nuclei for the oculomotor nerves. The present findings are based on experimental lesions of the third, fourth, and fifth cranial nerves in monkeys. Results demonstrate that the axons of small numbers of the cells of the mesencephalic nucleus do reach the extra-ocular muscles, and that they traverse the oculomotor trunk. Corbin's view that sensory neurones occur in the oculomotor nuclei is not supported by the evidence of this research.

Some observations on the distribution of cholinesterase in the spinal cord and hindbrain. By R. S. SNELL. *King's College, London*

Six cats were used for the investigation. The spinal cord and hindbrain of each animal were fixed in 10 % formol saline by vascular perfusion and by immersion for a total period of 24 hr. Frozen sections of each region were processed using the histochemical technique described previously.

In the spinal cord the enzyme activity was found to be greatest in the grey matter and was most active in the nerve cells of the lateral and anterior horns. In addition it was noted that the enzyme activity was greater in the anterior than in the posterior roots of the cord but in the posterior root ganglia a high activity was seen in the capsular cell cytoplasm. In the closed part of the medulla cholinesterase activity was greatest in the cuneate and gracile nuclei, the nucleus of the spinal tract of the trigeminal nerve and in the nucleus ambiguus. In the open part of the medulla and in the pons the enzyme was very active in the nuclei situated beneath the floor of the IV ventricle. In the cerebellum cholinesterase was seen to be very active in the granular layer of the cortex. The significance of these findings was discussed.

The correlation of structure and function in the postcentral gyrus of the macaque monkey. By T. P. S. POWELL and V. B. MOUNTCASTLE. *School of Medicine, Johns Hopkins University, Baltimore*

A combined histological and electrophysiological study has been made of the postcentral gyrus of the macaque monkey. Brodmann's original subdivision of this region has been confirmed. Anteriorly, area 3 is composed of koniocortex, and posterior to it areas 1 and 2 form a broad area of gradual change to typical parietal cortex.

The responses of single cortical cells evoked by mechanical displacement of skin and deep tissues have been recorded by microelectrodes. Cells have been found which could be excited by one of the following stimuli: movement of skin hair, skin pressure, movement of fascia, kneading of periosteum and passive joint movement. Most of the electrode penetrations which are perpendicular to the surface of the cortex are 'sub-modality pure'; in such a penetration all the responses are evoked by stimulation of either cutaneous or deep tissues. Functionally, just as structurally, there is an antero-posterior gradient of

change; anteriorly in area 3 more than 70 % of the cells are excited by stimulation of cutaneous receptors, but more posteriorly the proportions gradually change until 90 % of the cells in area 2 are related to receptors in deep tissues. The cytoarchitectonic subdivisions have been found to have a functional significance.

Chronological appearance of Nissl granules in cranial nerve nuclei
—Preliminary report. By G. T. ASHLEY. *University of Manchester*

Serial sections of brain stems have been examined in a series of six human foetuses (3–6½ months). Stains used—cresyl violet (2), gallocyanin (4).

Differences have been found in the time of development of Nissl granules in neurones of different nuclei within any particular functional group.

Somatic motor group (including branchial). At 3 months all nuclei contain neurones showing traces of granule formation, but at 4 and 5 months granules are more evident in V (motor), VII, X (nucleus ambiguus) and XII than in III, IV and VI.

Somatic sensory group. Granule formation is well shown in V (mesencephalic nucleus) at 3 months, VIII (vestibula) at 4 months, and to a lesser extent in V (main sensory and spinal) and VIII (cochlear) at 5 months.

These findings may be important in relation to Peterson and Murray's findings (*Amer. J. Anat.* 96, 1955) concerning the relationship of myelination to Nissl granule formation. So far, the investigation suggests early functional capacity of neurones concerned with the branchial musculature and the vestibular apparatus, as compared with neurones concerned with eye muscles, skin sensation and hearing.

The termination of primary vestibular fibres in the vestibular nuclei of the cat.

By D. BOWSER (*University of Liverpool*), F. WALBERG and A. BRODAL (*University of Oslo*)

Following destruction of the vestibular nerve in seven adult cats, preterminal and terminal degeneration in the vestibular nuclei has been traced by the methods of Glees and Nauta. There is complete concordance between the results obtained by each of the two methods.

In the superior vestibular nucleus, primary afferents are distributed mainly to the central parts of the nucleus, and end in synaptical contact with cells of all types. In the lateral nucleus of Deiters, vestibular fibres reach only the rostroventral regions of the nucleus; and the giant cells of this nucleus are almost entirely free from degenerating terminals. In the medial nucleus, contacts are made with cells of all types, but mainly in the lateral part of the nucleus. In the descending nucleus, terminal vestibular fibres are evenly distributed, except in the rostrolateral corner, which is relatively free; large cells in this nucleus are mostly free from degenerating terminals, and certain special subgroups, *f*, *x* and *z* (Brodal and Pompeiano, *J. Anat., Lond.*, 91, 1957) are also free.

These areas of termination interdigitate with the terminal zones of other afferent fibres to the vestibular nuclei. This pattern is described and the functional implications discussed.

Radiological examination of the vertebral and cranial venous sinuses in the monkey. By E. R. A. COOPER and J. B. D. TORR. *University of Manchester*

As part of an investigation into the cranial and vertebral venous sinuses, radiological examination was used. The cranial sinuses were demonstrated by taking serial radiographs during the injection of pyelosil into the common carotid artery of one side. In the case of the vertebral venous sinuses the method of trans-osseous phlebography was employed. The point of a sternal puncture needle was introduced into the body of a lumbar vertebra in an anaesthetized animal using fluoroscopic control. Injections of pyelosil were made through this needle and serial radiographs taken throughout the procedure. Examples of the results obtained were demonstrated.

Fat, muscle and bone in the limbs of young men and women: their quantitative interrelationships studied radiologically. By J. M. TANNER (*Institute of Child Health, London*), M. J. R. HEALY (*Department of Statistics, Rothamsted Experimental Station*) and R. H. WHITEHOUSE (*Harpenden Growth Study*)

The widths of fat, muscle and bone have been measured in soft tissue delineating X-rays of the upper arms, calves and thighs in 166 young women and 44 young men. Correlation coefficients have been calculated (for each sex separately) between the six resulting fat widths (over biceps and triceps, anterior and posterior thigh, lateral and medial calf), three muscle widths, and four bone widths (humerus, femur, tibia and fibula). The correlations between the widths of the *same* tissue at different sites are high or relatively so: the average intercorrelation between the six fat measurements is 0.67 for women and 0.79 for men (see Table 1 below); for the three muscle measurements 0.43 and 0.45, and for the three bone measurements (excluding fibula) 0.37 and 0.54. The correlations between *different* tissues however are virtually zero. The average of bone/muscle correlation is 0.13 for women and 0.03 for men; fat/muscle 0.09, 0.16; fat/bone 0.07, -0.02. The widths of humerus cortex and medulla have been separately measured. In a maximum likelihood factor analysis of all radiographic measurements three orthogonal factors, representing fat, muscle and bone, account for nearly all the variance, leaving very little for regional effects on more than a single tissue. It is noteworthy that humerus cortex saturates in the *muscle* and not the bone factor.

Table 1

	Fat	Muscle	Bone
Fat	0.67	—	—
	0.79	—	—
Muscle	0.09	0.43	—
	0.16	0.45	—
Bone	0.07	0.13	0.37
	-0.02	0.03	0.54

An introduction to human dissection. A film. By C. P. WENDELL-SMITH, D. B. LONGMORE and C. E. ENGEL. *Guy's Hospital Medical School, London*

The helpful preliminary instructions to dissectors which appear in some manuals are not in themselves sufficient introduction for the tyro. In a department with a low staff-student ratio, small group teaching is only feasible if it can be staggered; it is therefore an impracticable method of dealing with a large influx of new students when it is desired that they should all be introduced to human dissection simultaneously.

A film providing a visual and auditory aid to dissection has been made. By showing it to a large body of students much useful information may be covered systematically in a limited period of time; the delivery of similar material to small groups would necessitate prolonged and repetitive instruction.

The aim of the film has been to show *how* and not *what* to dissect, and to supplement the dissecting manual and orthodox teaching. It has been used with benefit for two successive intakes of students.

The film is shown and comment made on its value as a teaching aid.

Autografts of lining of urinary bladder to ear in rabbit. By J. JOSEPH. *Guy's Hospital Medical School, London*

Some skin was removed from the inside of the ear and a graft of the lining of the bladder (transitional epithelium and submucous connective tissue) was placed on the denuded area with the epithelium either outwards or on the graft bed. Dressings were applied and removed after 10 days. The ears were then left undressed and observed for periods of 20-100 days.

In many animals the grafts survived. In all of these some part of the transitional epithelium formed cysts. In the majority, some epithelium remained on the surface continuous with the skin epithelium at the periphery. Although these were usually infected, the transitional epithelium appeared to be healthy and frequent mitotic figures were seen. Muscle which was inadvertently grafted with the lining of the bladder frequently survived.

New cartilage and bone were also seen in the tissues near the graft. Bone appeared earlier and more frequently than in the ileal grafting experiments previously reported. Proliferation of the ear cartilage also occurred. In some animals it was difficult to distinguish between new cartilage which developed in the tissues of the new skin and proliferating cartilage of the ear.

Principles of bone repair. By J. J. PRITCHARD. *The Queen's University, Belfast*

A comparative study of the histological processes involved in bone repair shows that two primary activities, viz. the replacement of damaged tissues, tissue debris and blood clot by loose vascular connective tissue, and the formation of new bone on surrounding undamaged bony surfaces, are common to all. After very simple injury ossification spreads directly and rapidly into the loose connective tissue. After more complex injuries cartilage is formed, and the loose connective tissue tends to mature into dense fibrous tissue. In the first instance a type of cartilage with large closely-packed cells and scant matrix is formed from the proliferating osteogenic cells beneath the fibrous periosteum near the fracture line. After 'greenstick' fractures this is followed by chondrification of the outer part of the loose connective tissue—a fibro-hyaline type of cartilage being deposited. After complete sawn fractures the loose connective tissue matures into dense fibrous tissue before chondrification occurs and dense fibro-cartilage is formed. The periosteal cartilage is rapidly replaced; that formed in loose connective tissue more slowly, while replacement of fibro-cartilage is very slow and may fail.

Remodelling of the new and old bone begins well before bony union across the fracture gap and continues long afterwards. The general trend in remodelling is the replacement of fine cancellous woven bone by a cortical layer of lamellar bone enclosing one or more large marrow spaces.

Some aspects of the circulation in human bone. By T. E. BARLOW. *Department of Anatomy, King's College, University of Durham, Newcastle upon Tyne*

Specimens have been obtained by injecting lower limbs amputated in mid-thigh; the majority suffering from arteriosclerosis, but four from cases of Paget's disease of bone. Injection materials used have been neoprene latex, micro-opaque and colloidal silver iodide, and coloured gelatin solutions.

Examination of the material radiographically and microscopically suggests that Brookes and Harrison's conclusion (*J. Anat., Lond.*, 9, 1957) with regard to the blood supply of compact bone in the adult rabbit may also be applied to human bone, i.e. the principal supply to the cortex of the shaft is from the nutrient artery.

Two suggestions may be made with regard to the circulation in Paget's disease,

(1) The increased blood supply appears to be mainly through proliferation of the periosteal vessels.

(2) The disorganization of the circulation resembles that seen in tumour formation rather than that of any inflammatory condition.

The structure and distribution of primary and secondary ostons in certain adult mammalian long bones. By R. WALMSLEY and J. W. SMITH. *St Salvator's College, University of St Andrews*

In the development of mammalian long bones, primary ostons are formed about the time of birth. They appear in a matrix which consists in part of coarse fibred bone and in part of fine fibred surface lamellae. In Man, owing to the continual process of reorganization,

these three elements are largely, though not always, completely replaced by secondary ostones before adult life is reached; in the bones of certain mammals, however, the replacement is patchy, being well developed in some regions of bone and poorly developed in others.

In such adult animals, primary and secondary ostones have distinctive histological features. Their distribution within a bone can therefore be readily observed, and this distribution can be used as an index of the rate at which the process of reorganization occurs in different regions in the bone.

The submucosal vessel plexuses of the gastro-intestinal tract. By P. S. BOULTER (*Department of Surgical Studies, Middlesex Hospital, London*) and A. G. PARKS (*Department of Surgery, Guy's Hospital, London*)

Preparations of the submucosal plexus in various parts of the wall of the gastro-intestinal tract have been made. All of the specimens have been prepared from viscera removed at operation.

Intravascular fixation and staining has generally been used. Stripped and serially sectioned specimens have been examined. Observations have been made on the vessel plexuses in stomach, small and large bowel and rectum after these have been outlined by haematoxylin and silver. Of interest are the marked differences between the patterns of vessels in different organs and the specificity of pattern in any given viscus. The stomach has been noted to have a profuse plexus of vessels of all sizes while the small bowel has a close network of small vessels and the large bowel has a wider network of larger ones.

Arteriovenous communications have been noted in the submucosa, all of these being of the simple loop form described by Barlow. These have been assumed to be of the expanded capillary type previously noted in the mesentery.

Some clinical significance and application are discussed.

Changes in cytoplasmic concentration and mass during meiotic division.

By R. BARER and S. JOSEPH. *University of Oxford*

Measurements of cytoplasmic concentration were carried out by immersion refractometry on several thousand primary spermatocytes (from four species of insects) at different stages of division. The cytoplasmic volume was calculated from measurements of the cell and nuclear diameters, thus enabling the cytoplasmic dry mass to be deduced. In general there was a gradual fall in concentration throughout prophase, with a minimum just before metaphase. The cytoplasmic volume increased rapidly up to the pachytene stage, but more slowly afterwards. The cytoplasmic mass also increased rapidly up to pachytene but then tended to remain more or less constant until the disappearance of the nuclear membrane. The fall in concentration was much less than would be expected if the increase in volume were due to the imbibition of water alone. It appeared that active synthesis occurred from preleptotene to pachytene and this was followed by a relatively inactive phase during which some imbibition occurred. These results were discussed in relation to other work on cell growth and division.

The auditory apparatus of a foetus from a woman with two deaf-mute children.

By W. R. M. MORTON. *The Queen's University, Belfast*

A 48 mm. C.R. foetus was removed from a woman aged 34 years who, respectively eleven and six years previously, had given birth to a boy and a girl both of whom were deaf and dumb from birth. The foetus was fixed immediately after removal, bisected sagittally, and each half cut serially, one in the sagittal and the other in the transverse plane. No abnormalities were discovered in the brain, the 7th and 8th cranial nerves, the otic labyrinths, otic capsules, middle ears, ossicular chains and muscles, or in the tympanic

membranes and external meatuses; nor were abnormalities found elsewhere in the body. The chorion and other membranes were normal.

The labyrinth in mice with inherited nerve deafness is often apparently normal at an early foetal stage, but shows degenerative changes later in development. In human deaf-mutes changes in the organ of Corti are thought to be associated with prior changes in the area vasculosa. The chances of this foetus becoming a deaf-mute child were assessed, on genetic grounds, as one in four, so the specimen may not be genetically abnormal. The possibility that later degenerative changes would have occurred in the, as yet, undifferentiated area vasculosa cannot be excluded. Microscopic examination of many other genetically suspect specimens at later stages of foetal life are desirable, so that early changes in the auditory apparatus of potential deaf-mute children may be identified.

Thyroxine and hypervitaminosis A. By J. W. MILLEN and
D. H. M. WOOLLAM. *University of Cambridge*

The observations reported in this communication were in continuation of work previously presented to the Society on the teratogenic effects of hypervitaminosis A and the modulation of these effects by the concurrent administration of cortisone, insulin and 4-methyl-2-thiouracil.

Female rats of the Wistar strain were used in the experiments. Pregnancy was determined by the presence of spermatozoa in the vaginal smear. The pregnant animals were divided into two groups. Both groups received 40,000 i.u. vitamin A daily from the 8th to 13th days of pregnancy. One group received in addition 0.6 mg. thyroxine daily by subcutaneous injection from the 8th to 13th days.

All the animals were killed on the 20th day of pregnancy and the young removed from the uteri for examination. Only malformations of the head and cleft palates were considered in this communication. The incidence of malformations of the head was (7.1%) and of cleft palates (30.4%) in the 168 young from rats which received vitamin A alone. No malformations were observed in the 89 young from the rats which received thyroxine in addition to the vitamin A. These results supported the view that there existed an antagonism between thyroxine and vitamin A.

Observations on closure of the secondary palate in two strains of rat.
By J. W. S. HARRIS. *The London Hospital Medical College*

15- to 17-day-old embryos from forty rats of Chester Beatty and Lister Institute strains have been weighed and examined histologically and histochemically following fixation in six different fixatives. Litters with negative or minimal resorptions have been selected and of 448 embryos obtained, 125 have been serially sectioned either in a coronal or transverse plane.

In 15-day-old embryos of both strains the palatine shelves lie in a vertical plane close to the sides of the tongue except where they pass dorsal to its posterior extremity. The tip of the tongue separates the anterior ends of the shelves and lies in close contact with the ventral surface of the nasal septum.

Movement of the shelves dorsal to the tongue occurs during the 16th day, being earlier in Chester Beatty rats. It can be divided into stages similar to those described in mouse (Fraser and Walker, *J. Embryol. exp. Morph.* 4, 1956), but differing in that it appears to be initiated at the anterior ends of the shelves. No evidence of an elastic fibre network has been found in shelf tissue.

Shelf fusion is completed during the 17th day and ossification extends medially from the maxilla.

The blood supply of the diaphragm in the rat. By F. BECK and
J. S. BAXTER. *University College, Cardiff*

The distribution of the arteries and veins in the diaphragm of the rat has been studied by: (1) injection methods; (2) the staining of gross specimens (methods of Pickworth and Grant), and (3) the examination of microscopic sections.

The arterial supply is derived from (1) inferior phrenic vessels; (2) superior phrenic or pericardiaco-phrenic vessels; (3) intercostal vessels; (4) musculo-phrenic vessels, and (5) internal mammary vessels. The courses and areas supplied by these are described.

The venous drainage by anterior, middle, posterior—and accessory posterior—phrenic veins is found to be remarkably constant in its main features. The major veins lie just within the confines of the central tendon and show a bilateral symmetry. They represent the only tributaries of the embryonic hepato-cardiac channel (Streeter, *Cont. Embryol. Carneg. Instn.*, 1942, 30, 211) to be found in the adult. Two hypotheses, one genetic and the other mechanical, regarding the formation of these veins are advanced and discussed.

The presence of an anastomatic system of diaphragmatic veins connecting the inferior vena cava and azygos veins first noted in the cat by Sudzilovskii (*Ark. Gistol. I. Embriol.* 1956, 33, 43) is confirmed in the rat.

Lymphocyte production studied by means of tritium-labelled thymidine. By
J. M. YOFFEY (*Department of Anatomy, The University, Bristol*), N. B. EVERETT
(*Department of Anatomy, University of Washington School of Medicine*) and W. O.
REINHARDT (*Department of Anatomy, University of California School of Medicine*)

Tritium, the hydrogen isotope of mass 3, possesses two outstanding advantages for purposes of autoradiography. Its soft beta emission permits of excellent localization, while at the same time it inflicts the minimum of damage on living cells. Thymidine is believed to be incorporated specifically into newly formed DNA. Twelve male guinea pigs weighing about 400 g. have been given an intravenous or intraperitoneal injection of tritium-labelled thymidine (Schwarz) in doses of 1 μ c. per g. body weight, and at varying intervals ranging from 1 hr. to 8 days the thoracic duct lymph has been examined. The first labelled cells to appear are large and medium lymphocytes. Labelled small lymphocytes begin to appear at the 4th hour and then gradually increase until they finally form the great majority of the labelled cells. The data are interpreted as being in accord with the view that there are large numbers of newly formed small lymphocytes in the thoracic duct lymph of the guinea pig, and that they are released slowly from a pool of precursor cells in the lymphoid tissues. The findings are not regarded as consistent with the view that the thoracic duct lymphocytes are for the most part recirculating between blood and lymph.

Seasonal changes in the morphology of the human suprarenal cortex. By P. C. B.
MACKINNON and I. L. MACKINNON. *Royal Free Hospital School of Medicine and
King's College, London*

Hypertrophy and hyperplasia of the suprarenal cortex in response to cold are widely recognized phenomena in lower mammals and, by inference, are often held to be valid for Man.

As no data are available to support this thesis, suprarenal glands have been collected from a consecutive series of forty-four post-mortems at one London mortuary on women who had died suddenly during the luteal phase of the menstrual cycle. These have been divided into summer (April–September: twenty-one glands) and winter (October–March: twenty-three glands) groups.

Widths and nuclear densities of four cortical zones, z. glomerulosa, outer z. fasciculata, inner z. fasciculata, and z. reticularis have been measured in each gland and the summer and winter data compared.

Nuclear populations (zonal width \times nuclear density) are found to be increased in *summer*

in the z. glomerulosa due to an increase in nuclear density, and in the inner z. fasciculata due to an increase both in width and nuclear density. The nuclear populations of the outer z. fasciculata and z. reticularis are unaltered. Lipoid content in the four zones is the same in summer as in winter.

The implications are discussed.

The effect of limitation of movement of longitudinal muscle growth. By G. N. C. CRAWFORD, R. G. EDWARDS and A. B. ALDER. *Department of Human Anatomy, University of Oxford*

One hind foot of each of a group of young rabbits was immobilized in dorsi-flexion during a period varying from 4 to 25 weeks. This resulted in a considerable diminution in the longitudinal growth of the muscle belly of tibialis anterior. The muscle exerted isometric tetanic tensions almost as great as those of the control but at shorter lengths and through a reduced range of movement of the foot. Resting tensions of a magnitude similar to those of the control side were also developed at shorter belly lengths.

In a second group of young rabbits a chain joining the tibia to the foot allowed the latter to be fully dorsi-flexed but prevented plantar-flexion beyond an angle of 90° during periods extending up to 42 weeks. The effect on the tibialis anterior muscle was essentially similar to, although smaller than, that caused by complete immobilization of the foot.

The human temporomandibular joint disc. By A. D. DIXON. *University of Manchester*

Histological examination of post-mortem material (45–60 years) shows that the disc may be divided into two distinct parts: an anterior part composed of densely woven fibrous tissue and a posterior part of open texture characterized by the presence of vascular spaces. Cartilaginous changes are common in the fibrous part in older subjects. The posterior part of the disc contains a considerable amount of elastic tissue some of which forms a well-defined band passing from the posterior aspect of the fibrous zone to the squamo-tympanic fissure. This band may play a part in retrusive movements of the disc during jaw closure.

Bundles of myelinated and non-myelinated nerve fibres are found in the spongy tissue of the disc, frequently in relationship to vascular spaces. Nerve fibres are confined to the posterior part of the disc and the only nerve terminations seen have been of the freely ending variety. The nature of the innervation helps to explain joint pain in cases of compression of the disc following persistent overclosure of the jaws or in inflammatory conditions of the joint.

The submicroscopic components of developing dental enamel in the rat. By R. W. FEARNHEAD. *Departments of Anatomy and Dental Histology, The London Hospital Medical College*

Developing teeth from late foetal to 10-day-old rats, were fixed in Palade or Dalton's fixative, and embedded in methyl methacrylate or epoxide resin. In order to avoid the production of artefacts as much as possible, the material was not decalcified and sections were cut using a diamond knife. In addition fixed and unfixed fragments were dissected from the formative end of the incisors of adult rats, and mounted on perforated formvar or carbon films. The results indicated that the fibrous matrix was formed intracellularly and extracellularly. The extracellular matrix probably formed from a granular precursor which appeared earlier than the intracellular matrix between the formative ends of the ameloblasts. The transition between this granular material and the fibres was sharply demarcated. A further change from fibres into 'tape-like' structures took place, and this was associated with the onset of calcification. Using electron diffraction techniques, an attempt was made to gain information about the relationship between the organic and inorganic material present in this early stage of calcification.

The pattern of myoneural junctions in the developing tadpole of *Xenopus laevis*.By P. R. LEWIS. *University of Cambridge*

The pattern of motor innervation to various muscles in the developing tadpole of *Xenopus laevis* has been followed by a specially modified simultaneous coupling azo dye technique for cholinesterase (Lewis, *Quart. J. micr. Sci.* **99**, 1958) and by conventional silver techniques.

In many muscles, such as those of the eye and the developing hind limbs, the pattern is typical of higher vertebrates, that is, motor endings are distributed along the lengths of the muscle fibres with the majority concentrated into the central part of the muscle. In the axial musculature of the tail, however, myoneural junctions are confined to the region of the myocommata, and take the form of a 'cap' over the end of each muscle fibre. No evidence of normal endings along the length of these muscle fibres is seen. At first, endings in the abdominal musculature are also confined to the tendinous septa, but later in development the motor nerves ramify to form a typical pattern of innervation with endings distributed along the lengths of the muscle fibres.

In many muscles cholinesterase activity is retained at the ends of the muscle fibres after metamorphosis even though no motor innervation can be detected in this position.

Histogenesis of tail regeneration in the geckonid *Sphaerodactylus*.By ARTHUR HUGHES. *University of Cambridge*

In the regeneration of the lizard tail, it is well known that the full structure of the lost member is not restored. The axis of the regenerate consists of a cartilaginous tube enclosing a prolongation of the ependymal epithelium of the cord. The muscles are innervated by the last three pairs of spinal nerves of the stump. In the tiny West Indian geckonid *Sphaerodactylus*, the histogenesis of tail regeneration has been compared with the corresponding processes in normal vertebrate development. At the tip of the young regenerate, new cells proliferate from the surface of a sac of ependymal epithelium, and form a regenerative blastema, made up of cells of various potencies, from which cartilage, muscle, melanocytes and Schwann cells of the new tail are all derived.

Muscle fibres develop by the fusion of myoblasts, and the process continues into the functional period. The muscle bands of the regenerate become segmented into myotome-like divisions, and their innervation is derived by the penetration of nerve fibres from longitudinal trunks into the 'myocommata'. Within the muscle bands, a positive esterase reaction first appears in these intersegmental planes. In these respects, the neuromuscular development of the regenerate resembles the ontogeny of the axial musculature of an amphibian tadpole.

The development of the free end of the penis in castrated oxen.By R. R. ASHDOWN. *University of Bristol*

In a previous communication (*J. Anat., Lond.*, **91**, 580) it was shown that castrated oxen exhibited various degrees of adherence between penis and sheath. This was previously recorded by Mäder (1907) who believed that the more separate penises were also larger and possessed a better developed 'galea glandis'. To test this claim, the length of the free end and length of the galea glandis were measured on 207 penises from castrated oxen immediately after slaughter. The degree of adherence was classified by applying the criteria previously described (Class A, completely separate: Class D, almost completely fused).

The mean lengths (in mm.) were as follows:

Class	No. specimens	Length of free end	Length of galea glandis
A	43	75.4	22.6
B	46	70.2	21.6
C	64	66.4	22.1
D	4	63.4	22.3

Analyses of variance and χ^2 tests established dependence of length of the free end on class of adherence ($P < 0.01$) but for the length of the galea glandis this dependence was not established ($P > 0.2$).

The more separate penises were, in general, larger than the more adherent ones; this might be expected as separation and growth of the penis were both controlled by male hormone. However, the more separate ones did not possess a significantly longer 'galea glandis' and this agreed with the fact that the 'galea glandis' became relatively shorter in length during post-natal growth of the free end of the bovine penis (Ashdown, 1957, *J. Anat., Lond.*, **91**, 596).

Fixation of carbohydrates in the rat placenta. By D. BULMER and
A. D. DICKSON. *University of Aberdeen*

12-day rat placentae were examined in an attempt to assess the relative value of different fixatives in the preservation of carbohydrate. Each placenta was bisected. One half of each was fixed in 10% formalin, and the other in Carnoy's fluid, Bouin's fluid, acetic alcohol formalin or by the method of Lison and Vokaer (*Ann. Endocrin.* **10**, 66). Apart from the latter method, all fixatives were used both at room temperature and at 4° C.

Sections of each specimen were examined histochemically, and all showed the same qualitative distribution of glycogen. Though quantitative estimation was impracticable, glycogen was demonstrated in the giant cells, the junctional zone trophoblast and the decidua in apparently similar amounts with each fixative. The intracellular distribution of the glycogen varied considerably, however, according to the method of fixation. The distribution of diastase-fast PAS positive material did not appear to differ appreciably with different fixatives.

A case of isolated laevocardia with situs inversus viscerum.
By M. J. T. FITZGERALD. *St Thomas's Hospital Medical School, London*

The literature contains accounts of up to seventy cases of the above condition. The great majority of these are examples of transposition of the abdominal viscera alone. Only five autopsied cases are on record of transposition of all other viscera together with a left-sided heart showing internal transposition of its chambers. The present case belongs to the latter group, these 6 showing varied evidence of failure of torsion, or of countertorsion of the aortico-pulmonary septum during development.

In addition, the following television demonstrations were given by courtesy of Messrs Smith, Kline and French:

1. D. B. LONGMORE. Technique of dissection
2. P. L. WILLIAMS. Anatomy of the ear
3. W. C. OSMAN HILL. Some points in the comparative anatomy of living primates
(by courtesy of the Zoological Society of London)
4. P. S. BOULTER. The mesenteric circulation
5. E. W. BAXTER. Anaesthetization of lampreys
6. R. WARWICK. The visual pathway
7. D. B. LONGMORE. Abdominal and thoracic breathing
8. M. H. BISHOP. Facial musculature
9. J. P. WEAVER. Human heart, brain and living eye

FEBRUARY, 1959

An ordinary meeting of the Society for the Session 1958-9 was held on Thursday, 27 February, at the Anatomy Department, King's College, Strand, W.C.2. The President (Prof. F. GOLDBY) and the Vice-president (Prof. T. NICOL) occupied the Chair at the various sessions.

The following are the authors' abstracts of the papers presented:

Observations on the action of oestrogens on the phagocytic activity of the reticulo-endothelial system. By C. C. WARE. *King's College, London*

A kinetic method of estimating reticulo-endothelial activity employing particulate carbon was used in the present investigations which were carried out on 185 T.O. Swiss mice.

The effect of eleven non-steroid synthetic substances of varying oestrogenicity on reticulo-endothelial activity was assessed. The ability to induce stimulation of phagocytic function was found to lie parallel to the power of these substances to produce oestrus in the rat.

A detailed investigation of the dose-response relationship of the action of diethyl stilboestrol on phagocytic activity was carried out. It was found that the dose required to induce a significant stimulation of phagocytic activity was considerably higher than that required to produce oestrus in the mouse. At the level of administration necessary to produce increased activity other total body effects became pronounced.

The significance of the findings was discussed.

Age changes in the human suprarenal cortex. By P. C. B. MACKINNON and I. L. MACKINNON. *Royal Free Hospital School of Medicine and King's College, London*

Suprarenal glands were collected from fifty-nine men aged 20-86 years who died suddenly and the nuclear densities, widths and sudanophilia of four cortical zones, the z. glomerulosa, outer z. fasciculata, inner z. fasciculata, and the z. reticularis, were measured.

In the late decades nuclear density was significantly diminished and sudanophilia increased in each zone.

Total cortical width diminished to an insignificant extent in the late decades; however the inner z. fasciculata and the z. reticularis diminished significantly while the outer z. fasciculata increased significantly.

These changes were discussed.

Observations on islet-cell cytology in the pancreas of the normal and diabetic cat. By J. D. LEVER and MARJORIE JEACOCK. *Departments of Anatomy and Biochemistry, University of Cambridge*

Pancreatic biopsies were taken from adult male cats and, after a brief recovery period, daily injections of either growth hormone or crude anterior pituitary extract were started. Treatment was continued until a marked ketonuria, glycosuria, weight loss, polydipsia and a high D/N ratio denoted a severe metahypophyseal diabetic state: a further pancreatic biopsy was then taken. Biopsy material was fixed and processed for general and special (PAS and aldehyde fuchsin stains) histological examination and for electron microscopy. An unequivocal identification of α and β cells was possible by direct matching of light and electron micrographs from consecutive thick and thin sections: both cell types had distinctive fine structural features.

Richardson and Young have already described 'hydropic degeneration' of the β cells in the metahypophyseal diabetic dog. In a comparable diabetic state in the cat, the islets of Langerhans contained both normal and swollen α cells, but there were none of the typically-shaped β -cell granules in any of the electron micrographs. Many abnormal islet cells (probably β cells) appeared distended and exhibited large homogeneous areas: these cells were also found to contain large quantities of glycogen. These results were discussed.

A combined light and electron microscopic examination of the neural lobe of the pituitary of the rat. By A. HOWE (introduced by G. CAUSEY). *Royal College of Surgeons, London*

In a histological examination of the pars nervosa only blood vessels, unmyelinated nerve fibres and 'pituicytes' have been found.

The pituicyte is distinguished with both light and electron microscopes by the relative size of its nucleus ($4-9.5\mu$, long axis measurements) and by characteristic osmiophilic 'droplets' in its cytoplasm, also distinguished in the electron microscope by the relative density of its cytoplasm.

Two main types of cytoplasmic inclusion are preserved by osmium-containing fixatives:

(i) Relatively large osmiophilic 'droplets' (modal value 1.6μ range $0.8-4.8\mu$, by light microscope; modal value 0.85μ , range $0.4-1.6\mu$, by electron microscope) which, from electron micrographs, are clearly confined to the pituicytes.

(ii) Smaller-sized 'granules' (approximately $100\text{ m}\mu$ in diameter, by electron microscope), within the cytoplasm of nerve fibres, which are thought to correspond to the stainable 'neurosecretory material' (NSM).

The material of the pituicytes could be extracted from paraffin sections by turpentine, leaving NSM-staining apparently unimpaired. The complete lack of correspondence between NSM and material within the pituicytes suggests that the latter do not contain NSM.

The development of the common carotid artery.

By D. B. MOFFAT. *University College, Cardiff*

There is some difference of opinion concerning the embryological derivation of the common carotid arteries, some authorities stating that they are developed from the aortic sac or ventral aortic roots, and others that they are formed from the proximal part of the third aortic arch. A study of several hundred injected rat embryos suggests that the former theory is correct. In the rat, the third arch retains its ventro-dorsal direction much longer than it does in the human, and can be recognized long after the 'descent' of the heart. It will be shown that the external carotid arteries arise in the first place from the aortic sac and that the right and left horns of the latter become drawn out to form the common carotid arteries. The third arch thus forms only a small portion of the internal carotid artery, from the bifurcation to the site of the original attachment of the ductus caroticus. The position of the latter is demonstrated in a 14.3 mm. embryo in which the ductus caroticus had persisted.

A technique for the examination of ultrathin sections in the light microscope after electron microscopic examination. By A. A. BARTON. *Royal College of Surgeons, London*

In addition to the visual check between optical and electron microscopic pictures, it is also useful to examine the specimen itself after exposure to the electron beam. A technique has been evolved, therefore, for transferring the film to a microscope slide which forms a firm backing and at the same time a convenient base on which the sections can be stained and examined. After electron microscopy the grid was floated on the surface of a 3% solution of ferric chloride, copper side down. The copper was dissolved in 4 hr., by the reducing action of copper on a ferric salt, which was converted to ferrous chloride. This left the thin supporting film floating on the surface, and on top of this the sections; the ferric chloride was then carefully replaced with distilled water and the small circle of Formvar transferred to a slide. The methacrylate was dissolved by immersing the slide in carbon tetrachloride. The result of this processing was to leave thin sections lying on Formvar, which retained the markings of the grid bars, so that it was possible to identify any part of a section.

The majority of stains had no effect on ultrathin sections; however the sections were usefully stained with Unna's polychrome methylene blue for 24 hr.

Preliminary observations on freezing methods of fixation using the electron microscope. By B. A. YOUNG and F. R. JOHNSON. *The London Hospital Medical College*

In the present study of the fixation of tissues the electron microscope has been used for the evaluation of the results of different techniques. The findings with the electron microscope have been compared with those obtained with the light microscope following the treatment of similar tissues with the same methods.

Since it is generally agreed that optimum results are given by methods in which tissues are quenched at low temperatures and then either dried, substituted, or thawed, the present investigation has been concerned mainly with a comparison of these techniques. The influence of such factors in the techniques as size of tissue block, quenching temperature, substituting temperature, nature of substituting fluid and different thawing fluids has also been studied.

The results indicate that the electron microscope introduces new criteria for the assessment of methods of fixation and that the emphasis on certain aspects of the techniques may need to be altered.

A film of a dissection of the human middle ear.

By J. DUNCAN GRAY. *University of Sheffield*

A 16 mm. Kodachrome silent ciné-film of a dissection of the human middle ear, beginning with the removal of the tympanic membrane and ending with a display of the cochlea, vestibule and the lateral semicircular canal. By a photographic trick the process is then reversed to give the appearance of a reconstruction of the parts. Projected at 12 frames/sec., it occupies about 18 min.

Autoradiographic studies of the common bile and pancreatic ducts in rodents.

By R. M. H. McMINN and J. H. KUGLER. *University of Sheffield*

The terminations of the common bile duct in the hamster and rat, and of the common bile and pancreatic ducts in the guinea-pig, have been investigated by histochemical methods, supplemented by autoradiographic studies using sulphur-35.

In the hamster no glands are associated with the lower end of the bile duct, whose lining cells exhibit a PAS-positive border and supranuclear granules. There are many goblet cells. All these lining cells show strong radioactivity, suggesting that all are secreting sulphated mucopolysaccharides.

In the rat the lower end of the bile duct possesses few goblet cells and the columnar cells show no PAS-positive granules and only a faintly staining border. Simple tubular glands open into the duct and contain many goblet cells that exhibit strong radioactivity.

In the guinea-pig the common bile and pancreatic ducts open into the gut some distance apart. Both ducts are associated with many tubuloalveolar glands whose acini are packed with PAS-positive material that is strongly radioactive.

Thus while all the ducts mentioned secrete sulphated mucin, the manner of production shows distinct species differences.

The site of the salivatory nucleus. By C. C. D. SHUTE

and P. R. LEWIS. *University of Cambridge*

A modification of the Koelle technique for cholinesterases has been found to act selectively in rat on visceral efferent fibres. Serial frozen sections have been studied of the portion of the brain stem believed to contain salivatory centres.

The nervus intermedius is seen to emerge by two roots. Of these, the dorsal accompanies the vestibular root of VIII and the ventral is in series with the visceral efferent rootlets of IX. Both roots can be traced to a dorsally placed *salivatory nucleus*, in series with the Edinger-Westphal nucleus and the dorsal motor nucleus of X. The nucleus is subdivided by the anterior genu of VII into a larger lateral and a smaller medial part. It contributes to the visceral efferent outflow of IX, but there is no separate inferior salivatory nucleus. No salivatory cells occupy the traditional site for the superior nucleus in the lateral reticular formation. Many small cells rich in cholinesterase are scattered in the caudo-ventral pole of the main sensory nucleus of V. These neurons may have a reflex salivatory function.

A few root fibres of the nervus intermedius cross the midline. Most of the fine decussating fibres at this level travel within the descending limb of VII and appear, after crossing, to join the dorsal root of the nervus intermedius. They may form an efferent component of VIII.

Afferent fibres to the dorsal-medial thalamic nucleus in the cat.

By R. W. GUILLERY. *University College, London*

Degeneration in the afferent fibres to the dorso-medial nucleus has been studied by the method of Nauta and Gyax. Coarse fibres have been traced to the medial 3/4 of this nucleus from the medial septal nucleus, diagonal band and ventro-medial mid-brain. The mid-brain fibres ascend along the habenulo-peduncular tract and pass into the dorso-medial nucleus from its postero-dorsal aspect. The fibres from the septum travel through the rostral part of the medial forebrain bundle. From there some of the fibres take a direct course to the dorso-medial nucleus but others pass through the parataenial and lateral habenular nuclei before they enter the postero-dorsal parts of the dorso-medial nucleus.

In addition a group of fine fibres has been traced to the medial 1/3 of the dorso-medial nucleus; this degenerates after lesions in the nucleus accumbens and lateral sub-commisural region. It has not been possible to find fibres afferent to the lateral 1/4 of the nucleus.

There is no clear evidence that fibres from the hypothalamus pass directly to the dorso-medial nucleus. However, the fibres afferent to the nucleus appear to come from cells that lie on the afferent or efferent pathways of the hypothalamus.

An electron microscopic examination of the endoneurial cell.

By G. CAUSEY. *Royal College of Surgeons, London*

Complete transverse sections of the sural nerve of the mouse, the sural nerve and nerve to the medial gastrocnemius of the rabbit and the sural nerve of the rat have been examined in the electron microscope after fixation in buffered osmium tetroxide. If it is accepted that any cell whose cytoplasm directly enfolds myelinated or non-myelinated nerve fibres is a Schwann cell, then it will be shown that the majority of so-called endoneurial cells or endoneurial fibroblasts are Schwann cells. For instance in the sural nerve of the mouse, examination of the cellular topography connected with all the nuclei visible within the limits of the perineurial sheath shows that 85 % are Schwann nuclei, 10 % are endothelial nuclei. The 5 % remaining are not easily classified, but no cells that could be defined as fibroblasts are seen except possible rare examples in immediate relationship to a blood vessel.

Observations on the fine structure of Meissner's corpuscles. By NIKOLAJS CAUNA, and LEONARD L. ROSS.* *University of Durham, England, and Cornell Medical College, New York*

Meissner's corpuscle consists of a column of discoidal cells in parallel array with associated nerve endings following a meandering intercellular course. Electron micrographs reveal greater complexity in the form of the laminar cells than was previously envisioned.

* Supported by U.S.P.H.S. Research Grant B-1523 (C).

Mitochondria are numerous in the juxtannuclear region but relatively few in the more attenuated parts of the cells. The Golgi region is characterized by a profusion of minute vesicles. These are found in smaller numbers elsewhere in the cell where they resemble the synaptic vesicles of neurons.

The cells are separated by a thick layer of amorphous intercellular substance containing fine fibrils. In certain planes of section these run parallel and appear to be bound together by transverse bands of denser material that recur at approximately 1000 Å. intervals. Cut in other planes the fibrils form an hexagonal pattern reminiscent of that seen in Descemet's membrane.

The nerve endings exhibit an extraordinary concentration of mitochondria which vary markedly in size and internal structure. Some are particularly dense and are completely filled with closely packed concentric membranes that replace the normal pattern of cristae. Large laminated objects interpreted as myelin figures are also observed. The possible significance of these structural alterations in the axoplasmic organelles is discussed.

A simple general purpose tissue culture chamber.

By J. A. SHARP. *University of Leeds*

The chamber described is composed of a minimum of parts, all of which may be sterilized with dry heat at 150° C., and consists essentially of two coverslips separated by a layer of silicone rubber. When the explants have been inserted, the chamber is gripped firmly between two metal plates. Its chief advantage over existing apparatus of this type lies in the fact that after 24 hr. the plates can be removed and the coverslips will then remain permanently and firmly adherent to the silicone rubber. The subsequent absence of extraneous metal parts greatly simplifies microscopic examination of the cultures, which may be carried out with phase contrast optics using a conventional or an inverted microscope. In addition, the chamber is suitable for use with the interference microscope, and can readily be converted to a perfusion chamber by inserting two hypodermic needles.

A brief preliminary review is given of previous publications on this type of apparatus.

A case of a man with a uterus. By J. E. GRAY. *King's College, University of Durham, Newcastle-upon-Tyne*

This variety of sexual abnormality is the least frequently reported. Nearly all the cases are, like this one, found accidentally at operation. The body of the uterus was well developed, there was a cervix and a short vagina opening, presumably into the prostatic urethra. The testis was in the scrotum, the R. in the neck of the sac of an inguinal hernia. The end of the uterine tube formed the appendix of the epididymis. There was no ovarian tissue. The patient was of male nuclear sex. The microscopic appearance of the testes resembled that of XX chromosome cases of true hermaphroditism and of seminiferous tubule dysgenesis (Klinefelter's syndrome). The following theoretical conclusions are advanced: (i) The appendix of the epididymis is of Müllerian not Wolffian origin. (ii) The characteristic histology of the testis in cases of XX chromosome constitution is due to the lack of the Y chromosome. (iii) In this case the presence of a uterus indicates delayed differentiation of the testes in embryonic life.

Laminar destruction of the cerebral cortex for the study of retrograde thalamic atrophy. By L. KRUGER, L. I. MALIS and J. E. ROSE. *Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Md.*

A method for producing sharply limited destruction of individual laminae in the cerebral cortex of the rabbit and cat has been developed by employing fast particles of ionizing radiation (10 meV. protons and 20 meV. deuterons) delivered at a uniform energy. Using this method, it has been possible to destroy narrow bands of the individual laminae in

limited areas of the cerebral cortex, with apparently little damage to underlying and overlying layers. The thalamus has been examined for retrograde atrophy after destruction of each layer of the cerebral cortex. Preliminary results and possible applications are discussed.

Blood vessels of guinea-pig bone marrow. By J. M. YOFFEY. *University of Bristol*

The blood vessels of the bone marrow in the guinea-pig possess three distinctive features. The arteries, at first normal in structure, abruptly lose most of the tunica media and adventitia, and may run for a considerable distance with a wall composed of only two cell layers. The sinusoids possess a very thin but complete endothelium, which can be traversed readily by cells. Accumulations of small lymphocytes are often seen in the sinusoids, and this phenomenon is termed 'lymphocyte loading'. The veins are much larger in relation to the arteries than are veins elsewhere; this must mean a considerable slowing down of the blood flow through the marrow sinusoids. The veins are also unique in being remarkably thin-walled, the walls consisting of a single layer of endothelium as thin as that of the sinusoids. The significance of these structural features is discussed in terms of marrow function. There are no lymphatics present. Presumably the very thin endothelium of sinusoids and veins is freely permeable to all protein molecules, so that there is no difficulty in the return of extravascular protein to the blood-stream.

Sequelae of experimental partial ischaemia in long bones.

By M. BROOKES. *University of Liverpool*

The blood supply of the right hind limb in adult rabbits is partially suppressed by arterial ligation and section, in particular of the principal nutrient and popliteal arteries, so that the femur and tibiofibula are predominantly affected by the ensuing ischaemia, and peripheral gangrene did not occur. The left limbs have been used as controls. At weekly post-operative intervals the limbs have been injected intra-vascularly with 'Micropaque' and the diaphyses examined histologically and microradiographically.

The results to date show that in the presence of partial ischaemia, there is a periosteal arterial supply to cortical bone which is not evident in the controls. Histologically, apart from bone marrow necrosis, periosteal and endosteal new bone production occurs, with enlargement of the vascular spaces in the cortex; changes in the osteocytes are slight, and do not indicate massive cortical death.

The results are discussed in relation to the findings in human tibiae obtained from limbs amputated for peripheral occlusive vascular disease.

Age changes in the structure of secondary osteones in human bone.

By J. W. SMITH and R. WALMSLEY. *St Salvator's College, University of St Andrews*

In the present study of cortical bone from the human femur and tibia three types of secondary osteones have been noted. Type I is characterized by the regular alteration of lamellae containing predominantly circumferential fibres with others containing predominantly longitudinal fibres. In a type II osteone the longitudinal fibres resemble those in type I but the intervening lamellae contain only diffuse collections of fine collagen fibres. In a type III osteone lamellation is not an obvious feature because the longitudinal fibres predominate and the circumferential lamellae are thin and incomplete.

It is considered that the structure of an osteone tends to change as it becomes older. The pattern characteristics of type I and type II osteones tend to be replaced by those of the type III osteone. Moreover, it is suggested that the temporal relationship of type II and type III osteones may explain the age changes in the microradiographic appearances of osteones which have been described in recent years.

Is there a vestibule in the lung of *Gallus domesticus*?

By D. C. PAYNE and A. S. KING. *University of Bristol*

Nearly all accounts of the lung of *G. domesticus* agree that the primary bronchus increases in calibre soon after it enters the lung, so forming a distinct ampulla-like dilatation called the vestibule. From the vestibule arise the four ventromedial secondary bronchi. Caudal to the vestibule the primary bronchus tapers gradually, giving off the other three groups of secondary bronchi.

In 1943 Hazelhoff suggested that air circulates in the avian lung because of aerodynamic factors; these factors depended rather precisely on the curvatures and calibres of the primary bronchus and of the openings into it of the secondary bronchi. He confirmed this hypothesis by experiments with a glass model, which was meant to imitate the anatomy of the primary and main secondary bronchi as closely as possible. The model had a distinct vestibule.

We have looked for the vestibule in over fifty lungs of *G. domesticus*, using casts, bronchograms, and gross dissections. Our conclusion is that the classical vestibule is not present in this species. So Hazelhoff's model differs in this respect from the actual lung, the resultant discrepancies in curvatures and relative calibres being substantial. Therefore it is doubtful whether the results which he obtained from his model can safely be applied to *G. domesticus*.

APRIL, 1959

A meeting of the Society for the Session 1958-9 was held on Thursday and Friday, 16 and 17 April 1959 in the Department of Anatomy, University College, Gower Street, London, W.C.1. This was entirely devoted to a Symposium on 'The Ultrastructure of Cells'. The Chair was taken by the President, Prof. F. GOLDBY. The following papers were read:

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| 1. A. W. AGAR | Present and future possibilities of electron microscopy |
| 2. J. D. ROBERTSON | Techniques and principles of chemical fixation in electron microscopy |
| 3. M. C. LOW and
E. M. CROOK | Reactions of osmium tetroxide with amino acids |
| 4. F. R. JOHNSON and
B. A. YOUNG | Freezing methods of fixation |
| 5. H. E. HUXLEY | Sectioning and staining for the electron microscope |
| 6. A. A. BARTON | Replication techniques in electron microscopy |
| 7. M. S. C. BIRBECK | Electron microscope technique for macro-molecules |
| 8. H. LATTA | Rapid method for localization of tissue structure or lesions for electron microscopy |
| 9. J. Z. YOUNG | Electron microscopy and biological problems |
| 10. E. G. GRAY | Axo-dendritic and axo-somatic synapses of the cerebral cortex. |
| 11. H. LATTA | Aspects of the mechanism of fluid transport in renal tubules |
| 12. J. D. ROBERTSON | A molecular theory of cell membrane structure |
| 13. E. H. MERCER | Comparison of natural biological membranes with artificial models |
| 14. J. B. FINEAN | X-ray diffraction and electron microscope studies of the molecular structure of nerve myelin |
| 15. G. CAUSEY | The cell membrane and nuclear membrane of normal and experimental Schwann cells |
| 16. A. PETERS | The development of peripheral nerves in <i>Xenopus laevis</i> |
| 17. R. BARER, S. JOSEPH
and G. E. MEEK | Membrane interrelationships during meiosis |
| 18. G. E. PALADE | The secretory process in the exocrine cells of the pancreas |

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|-------------------------------------|---|
| 19. J. D. LEVER | Fine structural appearances in relation to function in certain secretory cells. |
| 20. A. D. HALLY | Electron microscopy of resting and active gastric parietal cells of the mouse |
| 21. R. W. FEARNHEAD | Secretory products of ameloblasts |
| 22. ET. DE HARVEN and
CHR. CÖERS | Electron microscopic observations on human neuromuscular junctions |
| 23. A. R. MUIR | Observations on the attachment of myofibrils to the sarcolemma |
| 24. J. D. LEVER and
P. DUNCOMB | The detection of intracellular iron in rat duodenal epithelium |
| 25. H. LATTA | Aspects of the mechanism of fluid transport in renal tubules |

The papers will be published separately in book form by Edward Arnold and Co., and the authors' abstracts are therefore not recorded.

In addition, there was a demonstration of electron microscope techniques with closed circuit television and panel discussion. The demonstration was conducted by Dr J. D. Robertson, assisted by the technical staff of the Department. The panel discussion was led by Prof. J. Z. Young—the panel consisted of Messrs H. E. Huxley, H. Latta, E. H. Mercer and G. E. Palade. The demonstration included the preparation of tissue for examination with the electron microscope and a demonstration of the Siemens Elmiskop 1 from the standpoint of its component parts and operation. The closed circuit television was arranged by the courtesy of Messrs Smith, Kline and French.

JUNE, 1959

The Summer Meeting of the Society for the Session 1958–59 was held on Thursday and Friday, 25 and 26 June 1959, in the Anatomy Department, Newport Road, Cardiff. The Chair was occupied at the various sessions by the President (Prof. F. GOLDBY) and Prof. J. J. PRITCHARD.

The following are the authors' abstracts of the papers presented.

A rare abnormality of the posterior vena cava in the dog. By F. BECK and J. S. BAXTER. *University College, Cardiff*

The posterior vena cava in the lower abdomen deviated to the lateral side of the right kidney, pierced the diaphragmatic right lumbo-costal trigone and in the thorax joined the azygos vein running its course to the anterior vena cava.

Renal, testicular, suprarenal and lumbar veins were dissected. A small vein lying dorsal to the aorta throughout its course was found to connect the azygos vein with the right postrenal vena cava.

The hepatic veins drained into the right atrium through a diaphragmatic opening in the position of the right hepato-cardiac channel.

A possible interpretation of the abnormality using the composite diagram illustrating the development of the mammalian posterior vena cava (Huntingdon & McClure, 1920) is that the 'supracardinal' vein in the lower abdomen (thoraco-lumbar line, para-ureteric segment) forms the definitive lower part of the adult vessel; this is continued to the lateral side of the kidney in the persistence of an anastomosis between the thoraco-lumbar and posterior cardinal veins passing ventral to the ureter (the common subcardinal vein draining the kidneys has migrated along this communication to a position *medial* to the ureter). The main vessel continues (representing the persistent posterior cardinal vein) through the lumbo-costal trigone in the diaphragm. It joins the thoracic azygos (medial sympathetic line) in the lower thorax.

It must be remembered that important differences in the details of caval development are found in various mammalian species (Butler, 1927) and therefore in the absence of a well documented study of the development of this vessel in the dog our theory as to the origin of the abnormality must be regarded purely as a suggestion.

Observations on the post-natal development of the mouse submaxillary gland.

By F. JACOBY. *University College, Cardiff*

The complex histological architecture of the adult gland is similar to that of rats previously described and histogenetically analysed (Jacoby & Leeson, *J. Anat., Lond.*, **93**, 1959). Acini, intercalated ducts, convoluted granular tubules and striated ducts have to be considered. For the study of their post-natal histogenesis glands of male and female mice of known ages from birth to six months were used. The 'mucoid' definitive acini develop only after birth from terminal tubules. The latter have relatively wide lumina and consist of cells filled with secretion granules which are PAS-positive and stain strongly with aniline blue in the azan stain. The growing acini gradually envelop remnants of the terminal tubules. Mode of acinar formation and fate of 'remnants' are discussed and compared with those of rats. Acinar development is practically complete between the 4th and 5th week; in the male somewhat earlier than in the female. Convolution of the intralobular ducts is evident from day 11; beginning granulation of these segments from day 24. In the male, this duct transformation is well advanced at 4 weeks, in the female not before 8-10 weeks. Further massive transformation of practically the whole intralobular duct system in male glands results, finally, in the well-known sexual dimorphism.

The development of the intra-arterial cushions in the arteries of the rat's eye.

By D. B. MOFFAT. *University College, Cardiff*

The arterial supply of the rat's eye is derived largely from a branch of the ophthalmic artery which divides into retinal and choroidal branches. Intra-arterial cushions composed of a tissue rich in acid mucopolysaccharides have previously been described (Moffat, 1956) in relation to the site of bifurcation.

At birth, the wall of the main vessel in the vicinity of the bifurcation is composed of a loose metachromatically staining tissue, the cells of which have spherical or oval nuclei. The wall of the artery is not sharply demarcated from the surrounding connective tissue. After a few days, smooth muscle fibres develop in the wall and isolate the inner portion of the metachromatic tissue which remains within the smooth muscle layer to form an intra-arterial cushion. By the end of the first week the cushion has become heaped up on either side of the orifice of the choroidal branch to form a pair of 'sentinel cushions'. By 4-6 weeks the cushions have become larger, more strongly metachromatic, and have reached their adult form.

Effects of parathyroid hormone and chondroitin sulphate on the connective tissue of the rat kidney. By J. FOURMAN. *University College, Cardiff*

Current theory suggests that the deposition of calcium which parathyroid hormone may produce in the kidney is related to changes in the connective tissue ground substance.

In 6 rats given repeated small doses of parathyroid extract there was an increase of metachromatic Alcian blue staining material, in the glomeruli and around the collecting ducts; the ducts contained small PAS-positive casts. In one rat given the largest amount of hormone, altogether 800 $\mu\text{g.}$, some casts contained calcium. These changes could be secondary to the increased urinary excretion of mucoprotein which the hormone produces. Therefore 12 rats were injected subcutaneously with small repeated doses of chondroitin sulphate. The kidneys of these animals also showed an increase of acidic mucopolysaccharides and PAS-positive casts in collecting ducts. There was no evidence of calcium deposits in

the kidneys. When urinary calcium excretion was increased in 12 rats by injecting calcium gluconate in addition to chondroitin sulphate, calcium was deposited solely in the basement membrane of the first part of the proximal tubule as in rats given gluconate alone, and not at the sites showing increased Alcian blue staining or in the intratubular casts.

It is concluded that some of the connective tissue changes induced by parathyroid hormone can be reproduced by injections of chondroitin sulphate, but whether they are related to calcium deposition remains an open question.

Vein-artery relationships at the pelvic brim and the initiation of varicose veins.

By T. HEATON WILLIAMS. *University College, Cardiff*

The causation of varicose veins is an ancient, important and unsolved problem, although several theories have been presented in attempts to resolve the mystery. One should distinguish between the true, *initiating* cause which by coming into play precipitates the effect, and *contributory* causes which merely predispose the patient towards the condition. From an anatomical point of view compression of the venous return channels from the lower limb is a factor of some interest and has attracted attention.

It is not disputed that a pelvic tumour may initiate varices by obstructing the flow of venous blood from the lower limbs. It is thought, as an extension of this special case, that the pressure of abdominal viscera including a loaded colon (Cokkinis, *Lancet*, 1, 1168, 1933), or of arteries at the pelvic brim (Williams, *Lancet*, in Press, 1959), acting together, resist venous flow in a similar manner to a pelvic tumour but with far greater frequency.

In plantigrade man the veins are liable to such pressure because neither viscera nor arteries tend to hang away from the pelvic brim veins and inferior vena cava.

Nineteen dissecting room specimens, and two fresh post-mortem specimens in the third decade, were examined bilaterally to find the principal sites where resistance to flow might be encountered. Three such places were found at the pelvic brim where arteries appeared to compress the veins to a varying degree.

It is stressed that so far no attempt has been made to separate quantitatively the effects of viscera and of arteries in impeding venous return from the limb.

Anomalous right subclavian artery in a young human foetus. By J. D. BOYD.

University of Cambridge

A 48 mm. CR length female human foetus in which the right subclavian artery arose from the aorta to the left of the left subclavian artery is described. The aberrant vessel passes dorsal to the oesophagus to reach the upper surface of the right first rib. The right vertebral artery is a branch of the right common carotid trunk which takes its origin from the upper surface of the aortic arch in the position normally occupied by the innominate artery. As the anomaly was identified before the sections were stained it was possible to impregnate them with silver (Bodian's technique) and to investigate the distribution of the principle pressor-receptor regions. No pressor-receptors could be identified in the wall of the anomalous subclavian artery. Such nerve-endings, however, are present in the course of the right common carotid trunk and on the origin of the right vertebral artery. These receptors correspond in structure with those found in the carotid sinus and the aortic arch, and also to those found at the point of bifurcation of the innominate artery in normal embryos and fetuses. It is considered that the findings support the concept of an association between the aortic arch arteries and pressor-receptor mechanisms.

Alkaline phosphatase in the adrenal gland. By B. F. MARTIN and

W. J. C. WILKINSON, *University of Sheffield*

The histochemical distribution of this enzyme has been studied in the right and left adrenal glands of the male monkey and in those of both sexes of the rat, mouse, hamster, guinea-pig, rabbit, cat and man. The 'azo-dye' as well as the 'cobalt-sulphide' technique

was used: in the latter method, a range of incubation periods from 5 min. to 2 hr. was employed for each specimen, which assists in evaluation of the precise localization and relative intensity of the reaction. Normally, the reactions in the right and left glands were of similar intensity, but it was confirmed that some species (e.g. mouse and hamster) show a sexual dimorphism in the reaction intensity. In all species, the cortex of the gland showed a reaction, the distribution and intensity of which varied with the species. The reaction was localized either in parts of the reticular network, the vascular endothelium, or in the cytoplasm of certain cells. The striking feature of the cytoplasmic reaction was the presence of an intensely positive paranuclear focus. Some species (man and monkey) showed a reaction in all three elements. Although reticular fibres and vascular endothelium reacted in the medulla of some species, the medullary cells were consistently negative.

A simple sulphation technique for the demonstration of tissue carbohydrates.

By T. A. I. GRILLO and P. R. LEWIS. *University of Cambridge*

A simple and convenient procedure has been devised for the staining of carbohydrate derivatives in tissue sections. Dewaxed sections are sulphated either by immersion in a 1:1 acetic-sulphuric acid mixture for 10–15 minutes or by exposure to sulphuryl chloride vapour for 15–20 minutes. The slides are then stained in a 0.05 % solution of methylene blue buffered to a pH of 2.5. Highly selective staining of tissue carbohydrates is obtained. The various steps in the technique are discussed, with particular emphasis on the reasons for the final choice of conditions. The sulphuryl chloride procedure is particularly useful for the study of glycogen which is intensely stained. After sulphation with the acetic-sulphuric mixture glycogen is not stained: otherwise the pattern of staining seen is very similar to that obtained with the PAS technique. These sulphation techniques have, however, several advantages over the PAS procedure: the overall picture obtained is clearer; definition at high magnifications is often better; and the versatility of the technique can be widened by varying the precise conditions of sulphation and staining to suit the particular problem being studied.

The ultrastructure of chromosomes and their behaviour in living cells.

By J. BOSS. *University of Bristol*

Any scheme of chromosome structure must be compatible with what is known of the behaviour of chromosomes in life.

When living fibroblasts of adult newts were observed in tissue culture, the transformation of the chromosomes at the end of mitosis could be followed in considerable detail. As the chromosomes separated in telophase each was connected laterally to its neighbours within the daughter group by fine bridges. After fixation these interchromosomal bridges were found to contain or consist of desoxyribonucleic acid. They persisted at least into early interphase.

Examination of the earlier stages of division indicated that the contact which preceded the drawing out of bridges between separating chromosomes could not have resulted from mere proximity but must also have required a change in the chromosomes themselves. The clumping of chromosomes which preceded bridge formation was itself preceded by the shedding of ribonucleo-protein from the chromosomes.

The following questions were discussed. What types of ultrastructure, compatible with existing knowledge, would permit part of the chromosomal desoxyribonucleic acid to be arranged non-axially? What type of ultra-structure would explain how the non-axial arrangement becomes first manifest when chromosomes are able to touch one another (or to come closer than is possible at other times)? Does our knowledge of the behaviour and structure of ribonucleic acid help in forming hypotheses to answer these questions?

The cytology of the pituitary gland of the female ferret. By R. L. HOLMES.
University of Birmingham

Six distinct types of cell can be demonstrated in the pars distalis of the pituitary gland of the female ferret, after staining by performic acid-Alcian blue-periodic acid-Schiff-orange G (Adams and Swettenham, *J. Path. Bact.* **75**, 1958).

Three of these are mucoid cells. The first contains cytoplasmic granules which stain with Alcian blue alone; the second contains similar blue-staining granules, together with a variable amount of PAS-positive material; while the third type contains only PAS-positive material.

The fourth cell type, apparently equivalent to the 'carminophil' in the cat and rabbit, stains deep orange due to a cytoplasmic affinity for orange G coupled with a faint positive PAS reaction. The fifth type is the ordinary acidophil, which stains with orange G alone, and the sixth the chromophobe.

The relationships between these various cell types, and their possible functional significance, are discussed.

The blood supply of the ferret's pituitary gland. By H. EVANS ROBSON.
Loughborough Training College

Owing to the common use of the ferret as a laboratory animal in pituitary research, its hypophysial blood supply has been studied. Berlin blue injections were performed post-mortem, with subsequent examination of dissections and thick serial sections. In half the number of animals injected, rice starch was added to prevent filling of vessels of under 20μ diameter. Although following the general mammalian pattern, details of vascular arrangements differ from those of other species studied by previous workers, and variations occur amongst individual ferrets examined.

The inferior hypophysial artery arises from the intra-cavernous part of the internal carotid artery, branches supplying the dorsum sellae and neural lobe. One branch runs over the neural lobe supplying it and usually anastomoses with a branch of the superior hypophysial artery. The superior hypophysial artery sends branches to the optic chiasma and pars tuberalis; from the latter arises the capillary network from which the portal vessels run. Another branch forms a collar around the dorsum of the stalk, and anastomoses with the inferior hypophysial artery. No arteries have been observed supplying the pars distalis, which obtains its blood via the portal vessels, and from wide-bore channels which enter it from the lower end of the stalk.

Mechanical factors in micturition. By S. A. VINCENT. *The Queen's University, Belfast*

In the living subject it has been found that the act of micturition can be stopped by upward pressure on the perineum *behind* the bulbar urethra, and furthermore that incontinence of urine can be relieved by special belts which produce this pressure.

Ciné-radiography during the act of micturition shows that there is a lowering of the bladder outlet just before it opens, and that sudden stopping of micturition is associated with raising this region.

In unfixed post-mortem bladders lifting the bladder neck closes the bladder outlet and stops the escape of fluid.

Patients with incontinence of urine associated with wide bladder neck can be cured either by operations which narrow the neck, or by 'sling' operations which render the levator ani lifting mechanism more efficient.

In order to find the correct degree of tightening at operation a periscope has been designed which permits a view of the bladder outlet from above under measured internal pressure. Preliminary investigations with this instrument show that pushing on the anal

region tends to close the bladder outlet, but that if this outlet is too wide at the start then closure is incomplete.

The role of this lifting mechanism and the importance of variations in width of bladder neck in normal and abnormal micturition are discussed.

Further studies of the venous sinuses of the central nervous system.

By J. B. D. TORR and E. R. A. COOPER. *University of Manchester*

Experiments were performed in an attempt to demonstrate the relationship between the cranial and vertebral venous sinuses. During the introduction of radio-opaque media into the vascular stream, the intra-abdominal and intra-thoracic pressures were varied and serial radiographs taken. The results obtained were demonstrated and discussed.

The comparative morphology of the enteric neurons.

By J. R. RINTOUL. *University of Manchester*

Various neurohistological staining methods have been employed in a study of nerve cell types occurring within the ganglia of the myenteric nerve plexus.

It was established that a classification of the neurons into different types was justifiable not only on morphological grounds, as described by Dogiel (1895, 1899), but on the basis of their different affinities for nerve stains. Thus (Dogiel) Types I and III neurons show a greater affinity for silver stains whilst those of the Type II variety exhibit greater electivity for methylene blue.

Futhermore, differences in nuclear staining were noted. For example, using a modified Bielschowsky technique, Type II neurons show a pale staining of the cytoplasm with a deep impregnation of the nucleus, whereas in Types I and III the cell body is heavily stained and the nucleus pale.

The distribution of these different neuronal types was also found to vary throughout the length of the digestive tract. In stomach of cat and guinea-pig, for example, Type II neurons alone are found. The diverse opinions existing regarding the function of these cells render the latter finding of some significance.

Finally, it was observed that the microscopic appearance of the enteric neurons was characteristic for animals of any one species.

Preliminary observations on the histochemistry of granular material in the human cerebellum, hypothalamus and medulla*. By W. M. SHANKLIN and M. ISSIDORIDES. *American University of Beirut School of Medicine*

Ten staining methods were applied to the human cerebellum, hypothalamus and medulla. (1) Periodic acid-Schiff was used for 1, 2-glycols, (2) alloxan-Schiff for proteins, (3) Sudan black B for fats, (4) luxol fast blue for phospholipids, (5) carbol fuchsin for acid fast material, (6) Gomori's chrome haematoxylin and (7) aldehyde fuchsin for neurosecretory material, (8) the Barnett-Seligman method for sulphhydryl groups of proteins, (9) Einarson's galloxyanin method was applied for the concurrent demonstration of RNA and acid fast material and (10) silver diamine for argentophilic material. Striking variations were found in the distribution of the above substances in the neurons, in the interstitium and in the walls of the blood vessels in the cerebellum, hypothalamus and the medulla.

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Histochemical localization of cholinesterase in the superficial receptors of the human finger. By N. CAUNA. *King's College, Newcastle upon Tyne*

Palmar digital skin obtained without anaesthesia from adult male was examined histochemically for acetyl cholinesterase and non-specific cholinesterase using Snell's modification of Koelle's histochemical technique. The tissues were fixed in neutral formalin for 3-6 hours, frozen sections were cut and incubated from 3 to 12 hours at pH 5.3 to 5.4.

It was found that in Meisner's corpuscles both acetyl and butyryl substrates gave strong positive reactions for the enzyme while the eserine controls gave no reaction. Nerve endings, nuclei of the laminar cells and the perinuclear cytoplasm seem to contain no enzyme when thin serial sections are examined under oil immersion; but the flattened parts of the laminar cells which are related to the nerve endings in a concentric manner stain strongly for cholinesterase even after a short period of incubation.

The so called 'free' endings in the dermal papillae also stain for cholinesterase, but it is difficult to define the precise cytological localization of the stain. The papillary blood vessels were entirely negative, and the sub-epidermal nerve plexus deep to the sweat ridges also showed negative reaction.

The significance of the findings is discussed in light of the ultrastructure of touch corpuscles.

Localization of cholinesterases in the forebrain of rat. By P. R. LEWIS and C. C. D. SHUTE. *University of Cambridge*

A modified Koelle technique for cholinesterases was applied to serial frozen sections of the diencephalon and basal telencephalon of the rat. The staining reactions were compared with those reported by Koelle (*J. comp. Neurol.* **100**, 1954) and various discrepancies noted, particularly in the thalamus and amygdaloid. Cholinesterase activity in the thalamus occurred mainly in the anterior nuclei (including the commissura inter-anterodorsalis but excluding the n. anteromedialis) and in the n. posterior. Cells of the n. anterodorsalis contained pseudocholinesterase. The (true) cholinesterase content of the amygdaloid was located in the lateral amygdaloid n. and the n. of the lateral olfactory tract. The following fibre tracts stained: fasciculus retroflexus (central fibres), dorsal division of the posterior commissure, anterior cingulum, ventral supraoptic decussation, and the so-called preoptic component of the stria terminalis, which could be seen to arise from the ventral n. of the lateral geniculate body. Other sites of true cholinesterase, in part at least intracellular, were the islets of Calleja, caudate-putamen, medial cells of the globus pallidus, n. accumbens, n. of the diagonal band, reticular n. of the thalamus, zona incerta, n. of the basal optic root, medial and lateral habenular n., n. parafascicularis and n. interpeduncularis. Cholinergic fibres appeared to end on the intercolumar tubercle (subfornical organ).

Dynamic changes at sensory nerve terminals. By M. J. T. FITZGERALD. *St Thomas's Hospital Medical School, London*

In the pig, Krause end-bulbs occur in the corium underlying epithelia supplied by somatic sensory nerve fibres, i.e. mucous membranes and non-hairy skin. A study of their structure and life history in the post-natal period is reported. There is evidence that the end-bulbs are a by-product of a continuous process of fragmentation and reformation of free nerve endings. Fibres which fail to enter the epithelium form end-bulbs by local Schwann cell proliferation. The bulbs are eliminated by autotomy of the parent fibres, with subsequent absorption by autolysis. These observations confirm and extend those of Oppenheimer, Palmer & Weddell (*J. Anat., Lond.*, **92**, 1958).

Studies on the formation of milk fat and milk proteins.By W. BARGMANN. *University of Kiel*

As revealed by light and electron-microscopic observations the formed elements of the milk consist not only of fat globules, with a diameter of $2-6\mu$ and ensheathed by a fine membrane, but also of small protein granules with a diameter of $40-120\mu$. It is not sufficiently clear by what means these particles are formed. So far it is generally assumed that in the process known as apocrine secretion superficial parts of the cells are pinched off into the lumen of the alveoli of the mammary gland. There are, however, no data regarding the formation of formed proteins.

Recently it was shown in electron-microscopic studies on the mammary gland of the rat, carried out in collaboration with A. Knoop, that in the cells of this gland two different processes take place at the same time: the first being concerned with the secretion of fat, the second with that of protein.

The fat is produced within the cytoplasm and concentrated in the apical parts of the gland cells in the form of small droplets. These droplets become ensheathed by a fine cytoplasmic membrane of about $17-29\text{ m}\mu$ thickness and are then given off into the acinar lumen. There are no indications whatsoever that the membrane could be made up of material originating from the plasma of the milk outside the cells. Independently from the production of fat droplets the protein granules are formed in the Golgi zone. It appears as if the protein particles are leaving the cell body through small holes in its membrane, but these holes are only transient features of the cell.

Studies on the myo-neural junction in amphibian myotomes. By B. MACKAY,A. R. MUIR, and A. PETERS. *University of Edinburgh*

In animals having a segmental arrangement of the trunk musculature, muscle fibres of the myotomes may receive nerves which envelop the ends of the fibres to form basket endings. These myo-neural endings are studied in four species of Amphibia by means of histochemical methods, by silver staining, and with the electron microscope.

Cholinesterase activity is found to be present as a diffuse cone over both ends of every muscle fibre. Nerves from the myocommal plexus approach the ends of the muscle fibres and ramify over their tips, the distribution of these nerves corresponding with the area of cholinesterase activity.

With the electron microscope, the terminal nerve filaments are seen to be unmyelinated axons applied to the sarcolemma between the clefts which the sarcolemma forms at the myotendinous junction. Each axon forms an expansion on reaching the sarcolemma, and vesicles present in the axon are more numerous in this expansion, particularly on the side nearest the muscle. There are no complex infoldings of the sarcolemma such as form the subneural apparatus in higher vertebrates.

The significance of double motor innervation of segmental muscle fibres is discussed.

Stria olfactoria mediana in the human brain. By G. BEAU.*Faculté de Médecine de Nancy***Fallacy of the 'open neural tube' hypothesis.** By J. A. KEEN.*University of Natal, South Africa*

A specimen of *total cranio-rachischisis* in a 7-month foetus was studied. The foetus was well-developed apart from the total destruction of the central nervous system. The *peripheral nerves* of the limbs were easily dissected and compared well with those of a normal 'control foetus' of the same age, in spite of the fact that the central nervous system had completely disappeared. The author believes that such an observation is incompatible with the 'open neural tube' hypothesis. The only form of pre-natal trauma which is recognized as being

able to destroy nervous tissue is the fluid pressure of an internal hydrops, and hydrops pressure can only arise in a closed neural tube with an already established cerebro-spinal fluid circulation.

The lesser congenital defects of this type, such as spina bifida, spinal meningocele etc., often show a normal spinal cord. If non-closure of the neural folds were the cause of such defects it would be necessary to postulate a secondary closure of the neural tube, after the defect had arisen.

In the surgical classification of these congenital posterior median line defects a two-fold aetiology is generally admitted: (a) some are said to be due to non-closure of the neural tube; (b) the majority are attributed to hydrops pressure of a disturbed cerebro-spinal fluid mechanism. The author concludes that the 'open neural tube' hypothesis should be abandoned as being fallacious and a hindrance to research in this field. It should be recognized that *all* such congenital defects are due to abnormalities of the cerebro-spinal fluid circulation.

The mechanism of wound contraction in rabbits. By M. ABERCROMBIE,
D. W. JAMES and J. F. NEWCOMBE. *University College, London*

The term 'wound contraction' describes the inward movement, as repair proceeds, of the intact tissue bordering a wound involving appreciable tissue loss. Its mechanism is a matter of current controversy.

In the experiments to be reported 2×2 cm. squares of skin were excised from each side of the thorax in a series of 15 rabbits. Contraction of the resultant wounds was prevented by Perspex splints glued to the surrounding skin.

The 30 wounds were divided into three equal groups. In the first (Group I) and the second (Group II) granulation tissue was biopsied, at 10 and 12 days respectively, with the splint in position. The central part of the granulation tissue was freed from its surroundings by an incision carried down to the subjacent panniculus. In both groups the granulation tissue islands thus isolated diminished in area and increased in thickness, while the peripheral margin of the incision retracted away from the centre of the wound. In the third group (Group III) splints were removed at 10 days, and granulation tissue biopsy, performed as before, postponed until the 12th day. Splint removal was followed by immediate wound contraction, biopsy by granulation tissue contraction.

Analysis of excised granulation tissue showed it to be of markedly similar composition in all groups in terms of collagen, water, nitrogen, and solid contents per unit wet weight. During rapid contraction, however (10-12 days, Groups I and III) there is considerable loss of substance from the wound, and likewise loss of collagen.

The significance of these findings for current theories of contraction is discussed.

A modulating influence of mesoderm on early trophoblast. By T. W. GLENISTER,
Charing Cross Hospital Medical School, London

Placentae of presomite and somite rabbit embryos have been grown as organ cultures either with or without the embryo and have been incubated for periods of up to 10 days. Oestrone, progesterone and mixtures of both have been added to the culture medium to ascertain whether they affect the activity of the trophoblast. This part of the investigation has so far proved negative.

If healthy foetal mesodermal elements survive in relation to the trophoblast, then it tends to remain organized into a syncytio-trophoblastic and a Langhans layer. This is irrespective of the fate of the embryo. When, however, the foetal mesodermal elements fail to survive, the trophoblast forms a hyperplastic plaque of cellular trophoblast. The two phenomena have been observed in the same specimen when the 'mesodermal backing' has been uneven.

These observations, considered in conjunction with the association in normal development of mesoderm with trophoblast, suggest that mesoderm exerts a reversible modulating

influence on early cellular trophoblast, inducing it to form syncytium and a Langhans layer.

In early stages, however, the trophoblast reverts to a primitive cellular variety when deprived of healthy mesodermal 'backing'.

The hypothesis may have relevance to the formation of trophoblastic cell islands and pathological changes in the human placenta.

Some observations on the placental giant cells of the rat. By A. D. DICKSON and D. BULMER. *University of Aberdeen*

Although never seen in mitosis and usually assumed to be polyploid, the placental giant cells form a layer which, remaining constant in thickness, grows in area with the enlargement of the placenta until the 12th day of gestation. The necessary increase in cell number is met by production from the junctional zone trophoblast. After the 12th day production stops but growth in area continues, with the result that by 17 days the placental giant cell layer is thin and even discontinuous.

Throughout the period under study the placental giant cells grow, the nuclei of the largest approaching one-tenth of a millimetre in length at 17 days. By this stage, however, their number is diminishing. Their incorporation in the 'capsule' (at which the placenta separates at term) is at least partially responsible for the decrease. During incorporation the nucleus undergoes a peculiar process of penetration by cytoplasmic ingrowths followed by fragmentation.

The evidence for the functions attributed to the placental giant cells is reviewed. It is shown that their phagocytic properties may be exercised only at certain stages and that they may also be involved in the elaboration of substances for secretion into the maternal blood stream.

The 'giant cells' associated with human placentation. By J. D. BOYD (*University of Cambridge*) and W. J. HAMILTON (*Charing Cross Hospital Medical School, London*)

The origin and distribution of the so called 'giant cells' of the human basal decidua and the adjacent myometrium have been studied in a spaced series of over 100 *in situ* human placentae. The specimens include stages from the 12th day after ovulation to full-term. Several post-partum uteri have also been available for investigation. The findings are interpreted as demonstrating a trophoblastic origin for these cells. In the stages of their maximum development the giant cells possess a quite remarkable structure and they can still be identified until, at least, four days after parturition.

Histological observations on the cutaneous glands in hippopotamus. By D. B. ALLBROOK, C. P. LUCK and P. G. WRIGHT. *Makerere College Medical School, Kampala, Uganda*

Peculiarities of certain obvious cutaneous glands of the hippopotamus are demonstrated.

These vary in size and position, but not in glandular components. They may be small (0.25 cm. in diameter), lying amid the matted collagen fibres of the dermis (e.g. in the ear pinna), or large (1-1.5 cm. in diameter) and flattened, lying just below the thickened collagenous dermis (e.g. over the flank).

The glands are tubulo-acinar in form and consist of two main categories of epithelia, namely granular and mucoid secretory cells. The mucoid cells contain secretion which is positive when subjected to Hale's colloidal iron technique, gives a positive periodic acid-Schiff reaction, and stains blue with Alcian blue. The granular cells are packed with coarse granules staining intensely with aurantia or acid fuchsin. No myoepithelial cells are found.

The glandular portion opens into a secretory duct which is of cuboidal epithelium. The larger secretory ducts are of stratified epithelium, with a thin outer coat of smooth muscle arranged as an helicoid.

Specimens taken at 10 hours after death show advanced cytolytic changes in the secretory ducts. This may perhaps be related to the character of the discharge from the granular cells which accompanies the tremendous post-mortem discharge of sweat.

Further studies of the salt-secreting ('nasal') glands of birds.

By R. J. SCOTHORNE. *University of Glasgow*

Previous studies of the nasal gland of the Aylesbury duck (*J. Anat., Lond.*, **93**, 1959 and *Quart. J. exp. Physiol.* **44**, 1959) described some histological and histochemical features of the inactive gland, and its extraordinary capacity of elaborating a practically pure and hypertonic solution of sodium chloride.

The main findings of the present study in ducks and pigeons are:

(1) A uniform abundance of succinic dehydrogenase in the secretory tubules of the inactive gland of the duck, correlated with the abundance of mitochondria.

(2) Reduction or absence of staining of mitochondria (Regaud fixation, acid fuchsin staining), and a corresponding reduction of mitochondrial phospholipid, during secretory activity in the duck.

(3) Relative paucity of mitochondria, and lack of functional activity, of the pigeon's nasal gland.

These findings are discussed in relation to the general problem of active transport of ions.

The chondrocranium of an embryo of the chimpanzee of 71 mm. CR length.

By D. STARCK. *Frankfurt-am-Main*

There is no information on the chondrocranium of the great apes. Pictures of a model of the cranium of a chimpanzee embryo (71 mm. CR length) are demonstrated. The base of the neurocranium between basion and anterior edge of the pituitary fossa is elongated. The facial axis (nasal capsule) is inclined in relation to the *basis cranii*. There is a large cranio-pharyngeal canal containing veins. The otic capsule is fused to the basal plate by anterior and middle basicapsular commissures. In *Pan* as well as in all other Primates studied—except *Papio*—an aliochlear commissure is missing. The dorsum sellae is large and perforated. The pronounced reduction of the lateral wall of the brain case and the asymmetry of persisting cartilages are remarkable. The reduction of the lateral wall of the chondrocranium in placental mammals is generally believed to be due to the progressive growth of the brain. Contrary to this assumption it can be shown that these correlations are more complicated. The evolutionary position of the species is also important in judging this question.

Pan has, like man, a short but distinct interorbital septum, the possession of which is generally taken as typical of monkey skull and explained by the influence of size and position of the eyes. Henckel states that an interorbital septum is an inconstant attribute of the simian skull. I found that the septum varied at different ages in many species. In young embryos of the howler monkey the septum is missing but is present in an embryo of *Alouatta* of 130 mm. length. It seems that the development of the interorbital septum depends on changes in the degree of declination of the skull base. Secondary cartilages are seen in the maxillary and pterygoid bones and in the lower jaw. There is great similarity in the foetal cranium of chimpanzee and the human skull. The differences are mostly of a quantitative nature. The fact is well known that the human and the pongid skull are more alike in young specimens. Thereafter development is different and divergent. Kummer has demonstrated that the dimensional relation between brain case and facial skeleton in the foetus and in adult man have their own laws of growth. The skull of adult man does not correspond with any one stage in the ontogeny of the ape.

Autoradiographic and alizarin techniques in the study of skull growth.

By A. D. DIXON and D. A. N. HOYTE. *University of Manchester*

The results of the two techniques, used quite independently, were compared to determine if morphological appearances were in agreement or at variance with each other. Many similarities were found in the corresponding regions in autoradiographs, which were prepared following the injection of radioactive calcium, and in sections of gross specimens of skulls of growing rats and rabbits stained with alizarin.

Regions compared included the cartilaginous sutures of the base of the skull, the fibrous sutures and bones of the vault, the olfactory fossa and para-condylar zone, and the tympanic bulla. In every instance the findings were in agreement, particularly in relation to the pattern of deposition and resorption in the growths of these regions. A striking feature was the persistence of resorption in certain of these areas, especially within the olfactory fossa and tympanic bulla, beyond the period described by many other workers.

It was concluded that autoradiographic and alizarin techniques are fully complementary, the results being further substantiated by those obtained by other histological techniques.

Features of facial growth revealed by radioactive calcium.

By A. D. DIXON. *University of Manchester*

Contact autoradiographs prepared from dried skulls and jaws of growing rats (birth-62 days), injected intraperitoneally with 2.5-10 microcurie/100 gm. body weight of radioactive calcium (^{45}Ca) and killed at variable intervals after injection, show accurate localization of the isotope in calcifying tissues within 30 minutes of injection.

In the maxilla and mandible, areas of pronounced subperiosteal deposition of ^{45}Ca on the facial and alveolar aspects are sharply contrasted with areas of relative inactivity on medial surfaces. A greater concentration of ^{45}Ca occurs in the zones of calcifying cartilage of the cranial base synchondroses and mandibular condyle, emphasizing the importance of these regions in increase in length of skull and mandible respectively.

In relation to the roots of cheek teeth, radioactivity of the bone forming the lingual wall of the dental sockets is greater than that on the buccal aspect of the roots, suggesting that as the jaws increase in width by subperiosteal deposition there is an associated outward movement of molar teeth.

The pattern of bone regeneration following limited experimental lesions in primates.
By D. B. ALLBROOK and W. K. CHAGULA. *Makerere College Medical School, Kampala, Uganda*

Colour illustrations were shown depicting the repair process following limited bone lesions of known size carefully made with a cooled dental fissure-burr.

Lesions were made in the parietal bones: (1) through the whole parietal bone, (2) through the outer table alone, (3) with periosteum intact and (4) with periosteum stripped and the surface for 1 cm. round the lesion coated with collodion or liquid Perspex.

Lesions were made in the tibiae as follows: (1) into the cortex, but not penetrating the medullary canal, (2) through the cortex into the medullary canal, and (3) total transverse fractures.

Certain facts emerging are worthy of note:

1. The poor osteogenic activity of the periosteal cambial layer in the cranial flat bones in cercopithecoids. Osteogenesis is more limited on the outer layer of periosteum than on the dural surface.

2. The total absence of cartilage production in both the skull and the comparable tibial lesions. This contrasts with the exuberant cartilage production characteristic of complete tibial fractures.

3. In the skull cortical bone production is mainly from osteoblasts forming a continuous

membrane with cells lining the diploic cavities. Ossification is pericollagenous and the resulting coarsely woven bone is 'plastered on' to existing bone surfaces.

4. Similarly in the limited tibial lesions new bone is formed by direct ossification around matted collagen connective tissue fibres.

The cross-sectional area of the intervertebral disc of the dog.

By R. N. SMITH and A. S. KING. *University of Bristol*

Some diseases of the vertebral column of the dog, especially those related to the intervertebral discs, show regional distributions. A series of measurements is being made to see if these distributions are related to physical factors. The cross-sectional area of the intervertebral disc is the first of this series and has been measured in 20 dogs of various breeds. The area is found to increase progressively from the first cervical to the last lumbar disc, except in the 'conjugal ligament region'. The latter is the part of the vertebral column where the discs are covered dorsally by conjugal ligaments which connect the heads of pairs of ribs: these discs are smaller than those on either side of the region. None of the distributions of disease processes seem to be directly related to the cross-sectional area of the intervertebral discs.

Marrow cellularity in different regions of the guinea-pig's skeleton.

By G. HUDSON. *University of Bristol*

In quantitative studies of haemopoiesis in the normal 400 g. guinea-pig, it is usually assumed that the cellularity (i.e. number of nucleated cells per unit volume) of humeral marrow is typical of that of the haemopoietic marrow as a whole.

In 16 guinea-pigs weighing 370–450 g., total nucleated cell counts were carried out on specimens of marrow obtained from sternum, ribs, humerus, femur and upper tibia, by the method described by Yoffey (*J. Histochem. Biochem.* 4, 1956). The mean values were 2.06, 2.01, 1.93, 1.83 and 1.78×10^6 per mm.³ respectively. The counts obtained from humeral marrow were significantly less than those obtained from sternal and costal marrow ($t = 3.1$ and 2.2 respectively) and significantly greater than those obtained from femoral and upper tibial marrow ($t = 2.4$ and 4.1 respectively). Whilst these mean differences did not exceed $\pm 8\%$, they must be borne in mind when humeral marrow counts are used to make approximate calculations of total numbers of marrow cells in the whole body.

It was also found that marrow cellularity decreased significantly with increasing body weight.

The results appear to reflect the fact that both local and general factors are important in determining the degree of activity in the haemopoietic marrow.

The fracture gap. By M. C. MULHOLLAND and J. J. PRITCHARD.

The Queen's University, Belfast

It is self-evident that the absolute width of the bony gap which must be bridged, and the nature of the tissues or materials occupying this gap, must be important factors governing the course and outcome of fracture repair. Nevertheless, the role of these factors has never been systematically investigated. In the present experiments using rats, 174 rib and fibular fractures of known initial width were studied radiographically and histologically. The widest gap which would be bridged with new bone 'unaided' was 1 mm. in the fibula and 2 mm. in the rib. Leaving a periosteal bridge in rib fractures enables gaps of 8 mm. to be united by bone; while a polythene tube crossing the gap and ensheathing the fractured ends allowed fibula gaps of 7 mm. to be spanned.

To minimize the possible effects of movement the repair of fractures in pieces of rib and fibula transplanted beneath the kidney capsule were studied. Fractured rib transplants united readily provided the gap was not more than 1 mm. wide.

It was concluded that only very narrow bony gaps can be bridged in the absence of (a) the means to keep adult tissues out of the gap, and/or (b) the presence of actively osteogenic tissue (e.g. periosteum) in the gap.

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